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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report contains the five manuscripts (one published, two in-press, one in-review, and one in-preparation) and six published abstracts completed during the three year grant period. These include descriptions of the four original research projects completed during the grant period. STUDY 1 was designed to test the Effects of illumination on human nocturnal serum melatonin levels and performance. Results indicate that overnight exposure to 300, 1500, or 3000 lux of light significantly diminished serum melatonin levels in a dose-dependent manner. Performance on vigilance, reaction time, and other tasks deteriorated throughout the night, consistent with known circadian variations in these parameters, but independent of ambient light intensity and circulating melatonin levels.

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18. Negative Pressure (LBNP), Melatonin, Mood, Performance, Pharmacology, Pulse Pressure, Reaction time, Sleep, Sleep Deprivation, Temperature, Vigilance

19. STUDY 2 was designed to investigate the Effect of pharmacological daytime doses of melatonin on human mood and performance. Results of this double-blind, placebo-controlled, repeated measure study indicate that 10, 20, 40, and 80 mg of melatonin, administered PO, significantly decreased oral temperature, number of correct responses in auditory vigilance, response latency in reaction time and feelings of vigor, relative to placebo. Melatonin also increased self-reported fatigue, confusion, and sleepiness. While areas under the time-melatonin concentration curve varied significantly in proportion to the various melatonin doses, performance did not vary in a dose-dependent manner. STUDY 3 was designed to investigate the Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. Very low doses of melatonin (0.1, 0.3, 1.0, and 10 mg, PO) or placebo were administered to 20 healthy male subjects during the study which used a double-blind, placebo-controlled, repeated measure latin-square design. Areas under the time-melatonin concentration curve varied in proportion to the different melatonin doses ingested, and the 0.1 and 0.3 mg doses generated peak serum melatonin levels which were within the normal range of nocturnal melatonin levels in untreated people. All melatonin doses tested significantly increased sleep duration and self-reported sleepiness and fatigue, relative to placebo. All of the doses also significantly decreased sleep onset latency, oral temperature, and the number of correct responses on the Wilkinson Auditory Vigilance task. These data indicate that orally-administered melatonin can be a highly potent hypnotic agent and suggest that the physiological increase in serum melatonin levels, which occurs around 2100 h daily, may constitute a signal initiating normal sleep onset. STUDY 4 was designed to investigate the Effects of L-Tyrosine ingestion during sustained simulator flight following sleep deprivation. Fourteen healthy male pilots ingested placebo or tyrosine (100 mg/kg of body weight) in a divided dose at 0100 and 0300 h during a simulated nighttime (2400 - 0800) cross-country flight, preceded by 19 hours of sleep deprivation. Analysis indicates that error levels were smaller when ingesting tyrosine, relative to placebo, on a significant proportion (0.79, $p < 0.001$) of the flight measures scored. The data further suggest that flight performance deteriorated between 2400 and 0400, the nadir of normal circadian rhythms, then improved somewhat after 0600 h. A similar circadian pattern was also found in oral temperature, reaction time latency and time-out errors, and self reported feelings of tension.

CONTENTS

CONTENTS (cont'd)

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Effects of Illumination on Human Nocturnal Serum Melatonin Levels and Performance

ANDREW B. DOLLINS,[1] HARRY J. LYNCH, RICHARD J. WURTMAN, MAE HUA DENG AND HARRIS R. LIEBERMAN[2]

Department of Brain and Cognitive Sciences, and The Clinical Research Center, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139

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DOLLINS, A. B., H. J. LYNCH, R. J. WURTMAN, M. H. DENG AND H. R. LIEBERMAN. *Effects of illumination on human nocturnal serum melatonin levels and performance.* PHYSIOL BEHAV 53(1) 153–160, 1993.—In humans, exposure to bright light at night suppresses the normal nocturnal elevation in circulating melatonin. Oral administration of pharmacological doses of melatonin during the day, when melatonin levels are normally minimal, induces fatigue. To examine the relationship between illumination, human pineal function, and behavior, we monitored the overnight serum melatonin profiles and behavioral performance of 24 healthy male subjects. On each of three separate occasions subjects participated in 13.5 h (1630–0800 h) testing sessions. Each subject was assigned to an individually illuminated workstation that was maintained throughout the night at an illumination level of approximately 300, 1500, or 3000 lux. Melatonin levels were significantly diminished by light treatment, $F(2, 36) = 12.77$, $p < 0.001$, in a dose-dependent manner. Performance on vigilance, reaction time, and other tasks deteriorated throughout the night, consistent with known circadian variations in these parameters, but independent of ambient light intensity and circulating melatonin levels.

Human Melatonin Mood Performance Reaction time Vigilance Sleep deprivation Fatigue Circadian rhythm

ENVIRONMENTAL illumination has been shown to be a prime factor in the establishment and maintenance of the rhythmic patterns of physical activity, core body temperature, and time of ovulation (9,11,12). These rhythms are correlated with release of the hormone, melatonin, which is secreted by the human pineal gland throughout the night, but not during the day (1,27,35). Environmental light is known to exert two effects on melatonin secretion:

1. entrainment of its endogenously driven rhythmic pattern with elevated levels occurring at night (27), and
2. acute suppression of its nocturnal elevation in response to light of sufficient intensity [i.e., 1500–2500 lux; (21)].

Melatonin has been shown, in a number of mammalian species, to serve as a Zeitgeber (or time giver) which entrains circadian rhythms. Rhythmic changes in serum melatonin levels may serve to couple various biological rhythms to the day/night light cycle (2,30,40). It might also be anticipated that changes in environmental circumstances which alter the normal light/dark cycle (e.g., transmeridian jet travel; shift work; space flight) would also alter the plasma melatonin rhythm, thereby altering the sleep/wake cycle and compromising performance.

Nocturnal bright light pulses have been used to explore the phase-response curve for entrainment of rhythmic melatonin secretion (21) and sleep/wake behavior (11). Such light pulses have also been used to treat a variety of maladies related to circadian organization (12,31). The fact that therapeutic efficacy typically requires repeated exposure to daily light pulses suggests that the light's action on the entrainment of circadian rhythms underlies its therapeutic effects. The significance of alterations in absolute levels of circulating melatonin has not been adequately examined.

The administration of melatonin during the day causes subjective sleepiness and induces sleep (23,37). This is an acute effect, not dependent on repeated doses, and may represent a direct effect of melatonin that does not involve alterations in the circadian pattern of its secretion. These observations have led us to postulate that the normal increase in nocturnal serum melatonin levels may have a direct influence on sleepiness and fatigue. In an attempt to isolate the effects of changes in absolute levels of circulating melatonin from the effects of its rhythmic occurrence, we sought to manipulate nocturnal melatonin levels experimentally and to assess resulting changes in mood, sleepiness, and performance. One of the fundamental tests of a pharmacologic agent on organic systems is the dose response. That is, if a compound has an influence on an organism, varying doses of the compound should cause corresponding variations in the response (assuming that the response plateau is not ex-

[1] Requests for reprints should be addressed to Andrew Dollins, Ph.D., MIT E18-473, 77 Massachusetts Ave., Cambridge, MA 02139.
[2] Current address: Military Performance and Neuroscience Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760.

ceeded). Lewy's observation (21) that acute exposure to a light intensity of 2500 lux totally suppresses nocturnal melatonin secretion, a lower dose of light (1500 lux) partially suppresses, and a still lower dose (500 lux) is without effect, suggests an approach to testing the relationship between endogenous melatonin levels and fatigue. If serum melatonin level does influence fatigue, as measured by mood and behavioral performance, then manipulating nocturnal serum melatonin levels by varying ambient light intensity should cause corresponding variations in fatigue.

The present study was conducted to test the hypothesis that performance changes observed under varying nocturnal light intensities would differ, and that these differences would parallel the light-induced changes in serum melatonin levels. A nocturnal working environment was simulated to test the practical relevance of ambient light intensity on performance.

METHOD

Twenty-four healthy male subjects (mean age = 23 ± 1.16 SEM, range: 19–39 years) participated in this study. Prior to admission to the study, each subject signed an informed consent form, had a physical examination to ensure that he was in good health, and completed two 1.5-h training sessions to familiarize him with testing procedures and the performance test battery. Subjects were also screened for depressive symptoms using the Hamilton Depression Rating Scale (18) with a special addendum for seasonal affective disorder (32). This protocol was approved by the MIT Clinical Research Center Advisory Board and Committee on the Use of Humans as Experimental Subjects. All subjects were paid for their participation in the experiment.

This study was implemented using a pseudoplacebo-controlled, repeated-measures, within-subjects, 3 × 3 Latin square design. Because the relative light intensity of a broad spectrum light may be discernible to subjects, a true placebo was not possible. Thus, a pseudoplacebo-controlled condition was devised by manipulating subjects' expectations. When subjects were initially briefed as to the purpose of the study, they were told that the study was designed to examine the effects of visual patterns and intensities of light on performance during sleep deprivation. In order to conceal the experimentally relevant stimulus dimension from the subjects, diffusing screens covering each light fixture were marked with horizontal, vertical, or diagonal lines (1 cm wide, separated by 2 to 2.5 cm). These lines had no effect on the individually adjusted illumination level of the lights. Because variations in spatial patterns are more perceptually salient than changes in illumination, subjects believed the pattern was the independent variable being tested. The line patterns were varied to counterbalance illumination level and test day. Throughout the study, the subjects questioned the investigators concerning the anticipated effects of the lines on the lights. They were asked to defer their questions until the study was completed.

The subjects participated in three 13.5-h (1630–0800 h) testing sessions, separated by at least 10 days, at the MIT Clinical Research Center. Upon admission, each subject was assigned to an individually illuminated computer workstation that was maintained throughout the night at approximately 300, 1500, or 3000 lux. The amount of luminous flux that each subject experienced was measured using a Tektronix J16 digital photometer with a J6511 illuminance probe. Frequent measurements were made to ensure illumination at the appropriate level. These measurements were made by holding the illuminance probe normal to the subject's line of vision at eye level. The light fixtures (Spectrum Industries, East Woodstock, CT) contained Philips FB40/CW/3 cool white fluorescent tubes. The light intensity emitted by each fixture was adjusted by varying the number of fluorescent tubes activated and by adjusting a rheostat that did not affect the spectral composition of the light.

On admission, a catheter with a heparin lock was established in a forearm vein for blood sample withdrawal. Blood samples were taken from each volunteer at 1900, 2100, 2300, and at hourly intervals thereafter until 0800 h. Serum was separated by centrifugation and stored at −20°C until assayed for melatonin concentration. Oral temperature, blood pressure, heart rate, and sleepiness were assessed hourly.

Throughout the night subjects were required to sit at their computer workstation, with eyes open, and to complete interactive computer tasks. They were not required to look directly at the lights and were allowed to vary their posture for comfort so long as this did not interfere with task performance. Two 20-min snack breaks (at 2400 and at 0400 h) and hourly toilet breaks (<5 min) were allowed. Subjects did not encounter light intensities greater than 300 lux during these breaks. The task order and times were held constant across test nights (see Table 1 for detailed schedule).

Melatonin Assay

Melatonin concentrations were measured in duplicate 1 ml samples of serum by radioimmunoassay (RIA) using CIDtech Ultraspecific Melatonin Antiserum (CID-tech Research Inc., Hamilton, Ontario) (7). The assay procedure is described in detail elsewhere (9). Briefly, 1 ml samples of serum were extracted into 5 ml of chloroform, the organic extracts were evaporated under a stream of nitrogen, and resulting residues were dissolved in phosphate buffer. Samples of the buffered extracts of serum were then analyzed by RIA. The sensitivity of the assay (defined as 3× the standard deviation of maximum binding) in the present study was 8 pg/ml.

Performance Test Battery

We selected performance tasks and mood inventories that previous studies have shown to be sensitive to the effects of melatonin, various hypnotics, other pharmacologic agents, and sleep loss (5,22,23,25,26). All performance tasks were administered on AT-class microcomputers equipped with hard drives and color VGA monitors. The computers were modified to port the speaker output to an audio amplifier (Realistic model: SA-150) equipped with stereo headphones (Realistic model: NOVA-40). To reduce the possibility of experimenter-induced bias, all tasks and instructions were automated by using a sequence of menus.

Mood and Sleepiness Measures

The Profile of Mood States [POMS; (28)] and Stanford Sleepiness Scale [SSS; (19)] were adapted to computer administration so the subjects could indicate choices by moving a cursor instead of using a pencil and paper. The SSS is a self-rated 7-point scale designed to quantify the progressive stages of the sleep–alertness continuum. The POMS is a self-report scale that consists of 65 adjectives, each of which is rated on a 5-point scale. Factor analysis yields the following scales: Tension–Anxiety, Depression–Dejection, Anger–Hostility, Vigor–Activity, Fatigue–Inertia, and Confusion–Bewilderment.

Dual Task

In this test, the subjects must simultaneously perform two tasks: a modified version of the Bakan Vigilance task (20) and the "estimation of two classes of events in a signal stream" (PROP) test (33). The Bakan Vigilance task presents, on the CRT screen, a sequence of three-digit numbers every 1.5 s for

TABLE 1

TIME LINE OF TESTING

1830	Subject Arrives
	Insert venous catheter
	Blood sample #1
1900	Begin baseline testing
	Profile of mood states
	Stanford Sleepiness Scale
	Simple Auditory RT
	Four-Choice RT
2000	Initiate Light Treatment
	Dual Task
	Digit symbol substitution
	Stanford Sleepiness Scale
2100	Blood sample #2
	Four-Choice RT
	Profile of mood states
	Stanford Sleepiness Scale
	Simple Auditory RT
2200	Dual task
	Digit symbol substitution
	Stanford Sleepiness Scale
2300	Blood sample #3
	Wilkinson Auditory Vigilance
	Stanford Sleepiness Scale
	Profile of mood states
2400	Blood sample #4
	Snack (20 min)
	Simple Auditory RT
	Stanford Sleepiness Scale
	Four-Choice RT
0100	Blood sample #5
	Digit symbol substitution
	Dual task
	Profile of mood states
	Stanford Sleepiness Scale
0200	Blood sample #6
	Wilkinson Auditory Vigilance
	Stanford Sleepiness Scale
0300	Blood sample #7
	Simple Auditory RT
	Dual task
	Four-Choice RT
	Profile of mood states
	Stanford Sleepiness Scale
0400	Blood sample #8
	Snack (20 min)
	Digit symbol substitution
	Stanford Sleepiness Scale
0500	Blood sample #9
	Wilkinson Auditory Vigilance
	Profile of mood states
	Stanford Sleepiness Scale
0600	Blood sample #10
	Simple Auditory RT
	Dual task
	Four-Choice RT
	Stanford Sleepiness Scale
0700	Blood sample #11
	Digit symbol substitution
	Profile of mood states
	Stanford Sleepiness Scale
0800	Blood sample #12
	Remove catheter
	Release subject

Task Name	Approximate Duration (min)	Number of Repetitions
Stanford Sleepiness Scale	1	13
Profile of mood states	5	7
Dual task	30	5
Wilkinson Auditory Vigilance Task	60	3
Four-Choice Reaction Time	5	5
Simple Auditory Reaction Time	5	5
Digit symbol substitution task	3	5

30 min. Usually, each successive number differs from the previous number by one digit. Occasionally, however, all three digits are repeated. The subjects' task is to detect the occurrence of these repeated sequences and to respond by pressing a key on the computer keyboard. Six blocks of 200 trials are presented during each test. Twenty-two three-digit number sequences are repeated during each block of trials. A single digit or letter is presented to the right of and simultaneously with the three digit number of the Bakan series. At the end of each block of trials, the subjects are required to estimate the proportion of letters (versus numbers) occurring in the last block of PROP stimuli. Subjects respond by moving a cursor on the computer screen to select a choice. The actual proportion varies randomly between 0.2 and 0.8, in increments of 0.1, for each block of 200 stimuli. The number of true positive and true negative responses as well as the proportions estimated and actually presented throughout each block of trials are retained in a computer file.

Wilkinson Auditory Vigilance Task

The Wilkinson Auditory Vigilance task is a computer adaptation of the task described by R. T. Wilkinson (38), with two modifications. Throughout the task, subjects must listen to a series of 500 Hz 70 dB (SPL) tones, presented at a rate of one tone every 2 s. The tones are of two lengths, long (500 ms) and short (variable ms). The subjects' task is to press a key when the short tone is heard. This version of the task is similar to Wilkinson's original version in that the tones were presented for 1 h

(1,800 tones) and 40 (2.2%) of the tones were short. Because the subjects were tested in a quiet room using headphones, no masking noise was used. In addition, the difficulty of short tone detection was evaluated and adjusted individually for each subject during training so each subject detected a short tone approximately 50% of the time (26). The length of the short tones ranged from 350 to 450 ms (mean and mode = 400 ms). Investigators monitored the subjects throughout administration of this task to ensure that they did not sleep or close their eyes. The number of true positive, true negative, and premature responses are retained in a data file.

Four-Choice Reaction Time (RT)

This test resembles the Wilkinson four-choice RT task and is a measure of visual vigilance (39). Subjects are presented with a series of visual stimuli at one of four adjacent spatial locations on a CRT screen. The subject must correctly indicate, by striking one of four corresponding adjacent keys on a microcomputer keyboard, the correct location of each stimulus. Four hundred trials were administered. The mean response latency and variances, as well as the number of true positive and true negative responses, are registered and retained. The number of premature responses (responses made during the 400 ms pause between stimuli) and time-out errors (failure to respond within 2000 ms of stimulus presentation) are also retained. Five warm-up stimuli and the response errors are not included in the total number of trials presented.

Simple Auditory Reaction Time

In this task, the subject responds, as rapidly as possible, to the onset of an auditory signal. The test trials are presented in rapid succession following five warm-up trials. A 300 ms 360 Hz tone warns the subject that a trial is about to begin. After a random delay of 100 to 900 ms, a 1000 Hz tone signals the subject to respond. Subjects are instructed to respond as quickly as possible after the onset of the 1000 Hz tone. The subjects' response latency appears on the computer screen for 300 ms between each trial. A warning message accompanied by an oscillating error tone occurs if the subject responds prematurely (prior to or within 50 ms of the onset of the stimulus tone) or fails to respond within 2000 ms. The subject must acknowledge premature and time-out errors by pressing the Enter key. A 1000 ms delay occurs between error acknowledgement and the next trial to allow time for the subject to reposition his hand. The response latency and variance, as well as the number of premature and time-out errors are retained in a data file. The task continued until 200 reaction times were recorded.

Digit Symbol Substitution Task

This task is a microcomputer implementation of the Digit-Symbol Substitution task of the WAIS-R Intelligence scale (14). It consists of a display of nine symbols in a row of boxes at the top of the screen, a display of nine corresponding numbers (i.e., 1 through 9) in a row of boxes below the symbols, a cue box and an answer box. The subject must use the numerical keypad to enter the number corresponding to the symbol that appears in the cue box, using the symbols and numbers at the top of the screen as a guide. The task is timed and the subject is instructed to enter as many answers as possible within 90 s. The first five symbols presented are considered practice trials and are not counted in the final output or in the timed portion of the test. The symbol associated with a given digit does not change within a 90-s set of trials but does change (randomly) each time the task is activated to prevent subjects from memorizing response keys. No response feedback is given to the subject, but a warning tone occurs if the subject presses a nonnumerical key on the keyboard.

Data Analysis

A repeated measures, within-subjects, 3 × 3 Latin square design analysis was used for all measures except serum melatonin levels. Repeated measures factorial analysis was used for serum melatonin levels because complete data was available for only 21 subjects. Group means were substituted for the performance data of one subject, who completed only one test session, in order to maintain the integrity of the study design for analysis purposes.

RESULTS

Serum Melatonin Levels

The mean serum melatonin levels of 21 of the subjects are illustrated in Fig. 1. The mean (±SEM) areas under the time-melatonin-concentration curve (AUC) between 1900 and 0800 h were 398.8 (±197.2), 288.5 (±121.4), and 247.6 (±125.2) under the 300, 1,500, and 3,000 lux conditions, respectively. Repeated measures factorial analyses of these values shows a significant light treatment effect, $F(2, 36) = 12.77$, $p < 0.001$. Contrasts indicated that the serum melatonin secretion under medium and high intensity lights was significantly lower than that under low intensity light ($p < 0.002$), and that serum melatonin secretion under high intensity light was significantly lower than that under medium intensity light ($p < 0.006$). A significant intraclass correlation (41), $F(21, 20, 42) = 3.741$, $p < 0.001$, $r_i = 0.477$, was found among the subject serum melatonin AUC measures, indicating reproducibility of melatonin response to light within subjects. The light treatment order and light treatment by light intensity effects were not significant.

Light Effects

There were no significant differences in performance associated with the three light treatment conditions. The Four-Choice RT correct response reaction time variance and number of time-out errors changed significantly with light treatment, $F(2, 42) = 4.162$, $p < 0.05$, and $F(2, 42) = 5.795$, $p < 0.01$, respectively. The mean correct response variances were 12895.7, 10471.3,

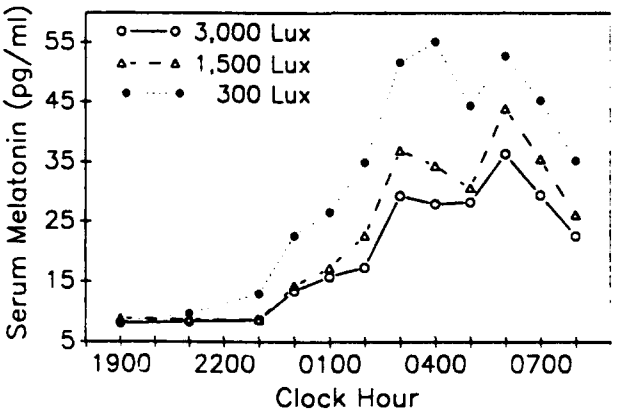

FIG. 1. Mean serum melatonin profiles of 21 subjects exposed to different light intensities and sampled at intervals throughout the night.

and 12609.7, and the number of time-out errors were 0.84, 0.37, and 0.60 for the 300, 1500, and 3,000 lux conditions, respectively. The greatest performance improvement in both measures was observed under the medium (1500 lux) light condition with the low- and high-light conditions being approximately equal. Initial analysis indicated a significant light treatment effect on the POMS Depression/Dejection scale. Examination of the data indicated, however, that the effect could be due to a baseline offset under the low light condition. Analysis of the data after removal of this offset (by subtracting the 1900 h value from subsequent scores) indicated that there was no significant light treatment effect on the POMS Depression/Dejection scale. There were no other significant light effects or light by time interactions on the performance or physiological measures. Results of a second analysis of the performance data from a subgroup of subjects with 3000/300 lux condition serum melatonin ratios of 0.6 or greater did not differ from those of the complete group.

Fatigue Effects

Overnight decreases in oral temperature (Δ, −1.29°F; Fig. 2) and heart rate (Δ, −8.66 bpm) were significant ($p < 0.01$). As illustrated in Fig. 3, mean responses to the SSS increased significantly from 2.28 at 2000 h to 5.29 at 0700 h, $F(2, 42) = 54.76$, $p < 0.001$, indicating an increase in sleepiness throughout the testing period. Similarly, all of the POMS scales showed significant (maximum $p < 0.02$) changes with time that are consistent with effects of circadian variation. Mean scores on the Tension–Anxiety, Depression–Dejection, Anger–Hostility, Fatigue–Inertia, and Confusion–Bewilderment scales increased by 1.43, 1.75, 2.39, 8.41, and 3.54 points, respectively, during the night. Mean responses on the POMS Vigor–Activity scale decreased from 16.99 at 1900 h to 8.49 at 0700 h. The average number of correctly identified Dual task target stimuli decreased significantly from 100.42 at 2200 h to 82.15 at 0600 h, $F(4, 84) = 20.671$, $p < 0.001$. Corresponding significant increases were observed, over time, in the number of stimuli incorrectly identified as targets, $F(4, 84) = 4.556$, $p < 0.002$, and estimates of the proportion of numbers versus letters presented, $F(4, 84) = 17.141$, $p < 0.001$. The average number of stimuli incorrectly identified as targets increased from 10.99 to 15.80, and the mean error in proportion estimates increased from 0.45 to 0.67.

Wilkinson Auditory Vigilance task responses reflected similar circadian effects. The mean number of correctly identified target stimuli decreased significantly, $F(2, 42) = 35.912$, $p < 0.001$, by 3.31 targets, and the mean number of premature responses significantly increased by 1.57 responses, $F(2, 42) = 4.266$, $p < 0.020$, as testing progressed throughout the night. The Simple Auditory RT mean response latency and number of premature responses increased significantly by 5.43 ms, $F(4, 84) = 7.109$, $p < 0.01$, and 2.84 responses, $F(4, 84) = 3.925$, $p < 0.006$, respectively, between the 2400 and 0800 h tests. Increases (maximum $p < 0.001$) were also observed in average latency (Δ +35.91 ms) and variance (Δ +87273.3) of Four-Choice RT task correct responses, as well as in the number of premature responses (Δ +1.76) and time-out errors (Δ +0.96), as testing progressed between 2100 and 0600 h. The mean number of correct responses on the Four-Choice RT task decreased from 375.65 to 361.53, $F(4, 84) = 9.774$, $p < 0.001$, during the same period. Some of the overnight performance decrements observed in the Four-Choice RT, Dual, and Wilkinson Auditory Vigilance tasks are illustrated in Fig. 4.

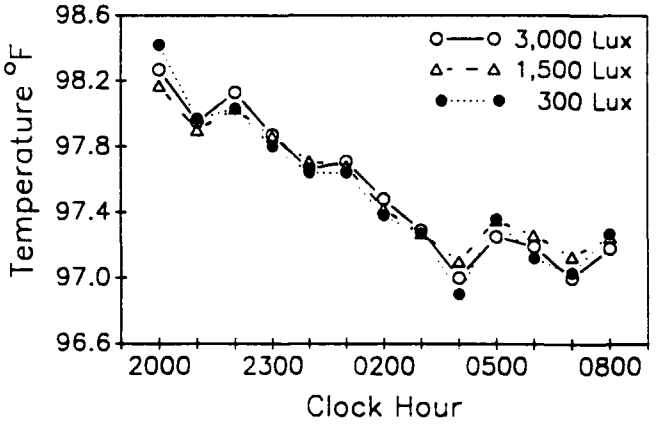

FIG. 2. Mean oral temperatures measured at intervals throughout the night during exposure to different ambient light intensities ($n = 24$).

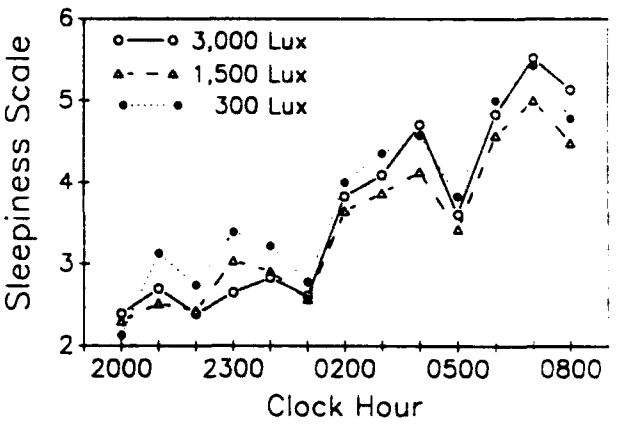

FIG. 3. Mean Stanford Sleepiness Scale responses assessed hourly in 24 subjects exposed to different ambient light intensities throughout the study period.

General performance decrements were observed on successive test days irrespective of light treatment condition. The number of Dual task correct responses decreased from 96.32 to 88.25, $F(2, 42) = 4.647$, $p < 0.015$, between test days 1 and 3. There were significant decreases in the mean number of correct (Δ −3.28), incorrect (Δ −59.57), and premature (Δ −2.57) Wilkinson Auditory Vigilance task responses on successive test days (maximum $p < 0.05$). Simple Auditory RT mean response latency also decreased from 177.04 to 167.67 ms over successive test days, $F(2, 42) = 19.179$, $p < 0.01$. Similar decreases were observed in the Four-Choice RT number (Δ −4.54), latency (Δ −12.46 ms), and variance (Δ −2028.01) of correct responses (maximum $p < 0.03$).

DISCUSSION

These data show that overnight exposure to ambient light intensities of 1,500 lux or 3,000 lux causes a dose-dependent reduction of nocturnal melatonin secretion compared to that observed at 300 lux (Fig. 1). However, parallel changes in the physiological and performance indices measured are not observed, even though some of the latter deteriorate significantly as the number of hours of nocturnal wakefulness increases. The changes observed in oral temperature (Fig. 2), the SSS (Fig. 3), the POMS Depression/Dejection and other mood scales, simple and choice RT and vigilance, are all consistent with previously

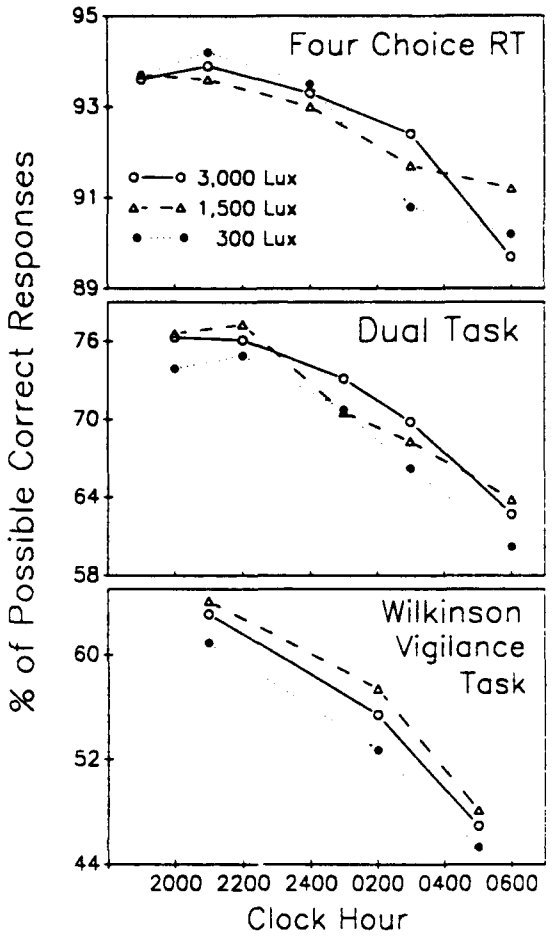

FIG. 4. Overnight performance decrement in the Four-Choice RT task, Dual task, and Wilkinson Auditory Vigilance task. The values plotted are average percent of the potential correct responses for each task under different light intensities (n = 24).

reported circadian variations in these parameters (16,17,19). Results of the current study differ from those reported in similar studies (10,15) which attribute performance changes to alteration in ambient light intensity. Among the factors which might account for this inconsistency are differences in the:

1. extent of melatonin suppression;
2. experimental controls; and
3. performance test batteries.

An interesting, though serendipitous, finding of the present study is the abrupt increase in oral temperature and decrease in circulating melatonin that accompanies a reduction in sleepiness following the 0400 h snack break (Figs. 1, 2, and 3). Decreased POMS Tension/Anxiety scale response levels were also observed between the 0300 and 0500 h test periods. The snack consisted of cheese, crackers, and noncaffeinated beverages. None of these substances are known, nor are they expected, to influence mood or melatonin secretion. It is likely that the observed mood and physiological changes were responses to the break in the testing routine rather than a direct effect of food consumption. It is interesting that the novelty of such a change in stimulation caused concurrent alterations in both mood and physiological measures. This phenomenon merits further investigation.

There were marked interindividual differences in nocturnal melatonin secretion. Total nocturnal melatonin secretion for the 21 men studied ranged from 153 to 1018 pg/ml/13 h (AUC) during exposure to the 300 lux condition. Total suppression of nocturnal melatonin secretion was approached in only a few subjects, even with exposure to 3,000 lux, the greatest light intensity studied. There was, however, a high level of intraindividual consistency in characteristic melatonin production, as evidenced by a significant intraclass correlation among individual serum melatonin AUC measures. This correlation occurred in spite of the systematic variation in total melatonin secretion induced by the light treatments.

The nocturnal melatonin levels observed under the 300 lux condition are approximately equal to those observed by Brainard et al. (6) in a similar study using a light intensity of 100 lux. Others have conducted studies which reported performance enhancement, although they employed the same or lower bright light treatments (10,15) and periods of relief from such exposure (4,10). These results suggest that differences in performance may not be attributable to either the intensity or constancy of light treatment. Suppression of nocturnal serum melatonin levels beyond those achieved in the present study may be necessary before behavioral effects are evident. Brainard et al. (6) found near total suppression of nocturnal melatonin secretion with overnight exposure to a light intensity of 3000 lux, an observation not confirmed in the current study. These inconsistencies indicate that a better measure of the light intensity actually impinging on the retina, or another property of the photic environment, is necessary to permit direct comparisons among studies of this nature (34). Precise quantification of the relationship between ambient light intensity, retinal illumination, and melatonin secretion would greatly facilitate our ability to assess the effects of light on human performance.

It is possible that sample size, experimenter and/or demand characteristic bias contributed to the differences between the findings of the current study and those of other investigators (10,15). For example, Campbell et al. cautioned that their performance results were based on a small subset of their subject population (n = 12; 4 in the 1000 lux condition versus 8 from the pooled 10 and 100 lux conditions) in which each subject was tested only once. In addition, subjects may have been tested by investigators who were aware of the expected results, in groups in which all of the subjects receive the same treatment simultaneously. It is, of course, very difficult to control for unintentional experimenter and demand characteristic bias under such a regimen. The influence of demand characteristics on reading performance and self-reported arousal was demonstrated by Veitch, Gifford, and Hine (36) who found differences in these measures resulting from positive or negative information given subjects regarding full-spectrum lighting effects.

Several controls were incorporated into the design of the present study to reduce the possibility of experimental bias. Lines on the diffusing screen of each light fixture served to confuse the subjects' perception of the independent variable being tested. Simultaneous testing of subjects under more than one condition and the use of menus to automate the testing sequence reduced the possible effect of demand characteristics. Repeated testing of the same, relatively large, group of subjects under all conditions reduced the between-group variance.

Some tasks in the current test battery differed from those used by other investigators (4,10,15). The test battery used in the current study centered primarily around mood, reaction time, and vigilance tasks. These tasks have all been shown to be sensitive to the effects of exogenous melatonin (13,23). All of these tasks proved sensitive to circadian variation but not to significant alterations in nocturnal melatonin levels. Neither French et al.

nor Campbell et al. reported statistically significant changes attributable to circadian variation in performance. The fatigue and circadian variations in performance observed in the present study may have been so large that they masked and/or obscured temperature and performance changes attributable to alterations in serum melatonin concentrations. The relative contribution of sleep loss, work load, and circadian variations in performance parameters (which may involve circulating melatonin levels) is a basic problem which remains to be resolved (3).

In summary, the results of this study indicate that the ambient light intensities tested did differentially suppress nocturnal serum melatonin secretion but did not cause corresponding differential alterations in the physiological measures or performance tasks examined. Changes in the behavioral parameters indicate a significant decrement in performance over time, consistent with previously reported circadian variation (16,17,19). Although our findings differed from those reported in similar studies (4,10,15), there are factors which bear further examination that could account for these conflicts such as differences in experimental controls, light intensities, extent of melatonin suppression, specific performance tests, and subject populations. This approach to testing the effects of diminished endogenous melatonin is necessarily limited to nocturnal investigation. The effects of sleep deprivation are, thus, inescapable concomitants of melatonin suppression. These effects may have masked any behavioral effects caused by suppression of nocturnal pineal melatonin secretion.

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Effect of pharmacological daytime doses of melatonin on human mood and performance

Andrew B. Dollins*, Harry J. Lynch, Richard J. Wurtman, Mae Hua Deng, Karl U. Kischka, Ray E. Gleason, and Harris R. Lieberman**

Department of Brain and Cognitive Sciences, and The Clinical Research Center Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139, USA

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Abstract. Melatonin (10, 20, 40, or 80 mg, PO) or placebo was administered at 1145 hours on five separate occasions to 20 healthy male volunteers and the effects on serum melatonin levels, mood, performance, and oral temperature were monitored. Subjects were studied between 0930 and 1700 hours. A battery of interactive computer tasks designed to assess performance and mood was completed, oral temperature was measured, and blood samples were taken for serum melatonin radioimmunoassay. The areas under the time-melatonin concentration curve (AUC) varied significantly in proportion to the various melatonin doses. Compared with placebo treatment, all melatonin doses significantly decreased oral temperature, number of correct responses in auditory vigilance, response latency in reaction time, and self-reported vigor. Melatonin also increased self-reported fatigue, confusion, and sleepiness.

Key words: Human – Melatonin – Mood – Performance – Reaction time – Vigilance – Fatigue – Circadian – Sleep – Alertness

Despite the human pineal gland's unique characteristics, particularly its rhythmic, photically entrainable secretion of melatonin at night, no function has been clearly associated with either the pineal or melatonin. Likely candidates for such a function include: participation in the regulation of circadian rhythms (Armstrong et al. 1986; e.g., human core body temperature; Lewy et al. 1991; Cagnacci et al. 1992); involvement in the production of sleepiness (Vollrath et al. 1981); humoral communication of information about environmental lighting (and thus time of day) to the brain and other organs (Reiter 1991); and determination of onset of puberty (Waldhauser et al. 1984). Melatonin is one of the few lipid soluble hormones and readily crosses from the circulatory system into all areas of the brain (Wurtman et al. 1968). The involvement of melatonin in animal physiology and behavior (particularly that associated with photoperiodism, or seasonality) is well established through such classic endocrinological techniques as surgical removal of the hormone's source (pinealectomy) and restoration of the hormone by administration of exogenous melatonin (Reiter 1988). Fully comparable studies have not been conducted in work with humans.

The hormonal message mediated by melatonin may be encoded in the rhythmic occurrence of melatonin in the circulation and/or the magnitude of the melatonin pulse. Human studies have shown that the daily administration of melatonin exerts physiologic effects, including: 1) feelings of fatigue (Arendt et al. 1984); 2) shift in phase response curve of rhythmic melatonin secretion (Lewy et al. 1992); 3) re-entrainment of circadian rhythms following a time zone shift (Arendt et al. 1986; Petrie et al. 1989; Claustrat et al. 1992); 4) subjective enhancement of sleep quality (MacFarlane et al. 1991); and 5) entrainment of previously free-running melatonin rhythms in blind subjects (Sack et al. 1991). These studies suggest that the efficacy of daily doses of melatonin may relate to the normal rhythmic occurrence of the hormone in the circulation. The behavioral effects of acute melatonin administration deserve further investigation.

Studies in humans show that acute melatonin administration increases subjective feelings of fatigue and causes decrements in performance. We have shown that a relatively large acute dose of melatonin administered at mid-day (240 mg, PO over a 2-h period) increases reaction time and induces drowsiness and sometimes sleep (Lieberman et al. 1984a). Nickelsen et al. (1989) found that an acute dose of melatonin (50 mg, PO) increased subjective feelings of fatigue when given in the morning (0900 hours), but not in the evening (1900 hours). Morton

Permanent address: *Permanent address: DoD Polygraph Institute, Building 3195, Ft. McClellan, AL 36205, USA

Permanent address: **Permanent address: Military Performance and Neuroscience Division, US Army Research Institute of Environmental Medicine, Natick, MA 01760, USA

Correspondence to: R.J. Wurtman

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and Naik (1989) report a significant reduction in diastolic blood pressure following acute administration of 25 and 50 mg but not 5 or 12.5 mg PO of melatonin at 0800 hours. Intranasal administration of 1.7 mg melatonin in the morning was shown to induce sleep onset (Vollrath et al. 1981). James et al. reported an increase in rapid eye movement (REM) latency following nocturnal melatnin administration (5 mg, PO) to normal subjects (1987) and as little as 1 mg, PO to insomniacs (1990).

The efficacy of acutely administered melatonin appears to be dependent on the magnitude of the dose and the time of administration. In the available literature on acutely administered melatonin, the smallest dose required to induce subjective and/or behavioral effects increased serum melatonin levels 10- to 100-fold above nocturnal endogenous levels, and thus represent pharmacologic doses. The behavioral effects of varying acute pharmacologic doses of melatonin have not been examined in a systematic manner.

This study was conducted to determine whether lower acute doses of melatonin than have previously been investigated have sedative-like effects on behavior, and if such effects are dose related. Various pharmacologic doses of melatonin were ingested at mid-day, when endogenous melatonin levels are minimal. A battery of interactive, computer based performance tasks were administered repeatedly to assess melatonin induced alterations in performance. Changes in serum melatonin concentration, mood, sleepiness, and oral temperature were also monitored throughout the day.

Materials and methods

Twenty healthy male subjects (mean age = 25 ± 1.47 SEM, range, 19–39 years) participated in this study. Prior to admission to the study, each subject gave his informed consent, had a physical examination to ensure he was in good health, and completed two 1.5-h training sessions to familiarize him with testing procedures and the performance test battery. Subjects were also screened for depressive symptoms using the Hamilton Psychiatric Rating Scale for Depression (Hamilton 1967) with a special addendum for Seasonal Affective Disorder (Rosenthal et al. 1987). The protocol was approved by the MIT Clinical Research Center Advisory Board and Committee on the Use of Humans as Experimental Subjects. All subjects were paid for their participation in the experiment.

The study was double-blind and placebo controlled. A repeated measures, within subjects, 5 × 5 Latin Square design was employed. The subjects participated in five 7.5-h (0930–1700 hours) testing sessions. At least 5 days elapsed between successive test sessions. Capsules containing 10, 20, 40, or 80 mg melatonin or placebo were administered (PO) at 1145 hours each test day. Treatment order was determined by a balanced Latin Square design.

On admission, a catheter with a heparin lock was implanted in a forearm vein for blood sample withdrawal. Blood samples were taken from each volunteer at 1000, 1200, 1300, 1400, 1500, 1600, and 1630 hours. Serum samples were separated by centrifugation and stored at −20°C until they could be assayed for melatonin concentration. Oral temperature, blood pressure, heart rate, and sleepiness were assessed hourly. Digital oral thermometers were

Table 1. Time line of testing

0930 Subject arrives
- Insert catheter
- Blood sample #1

1030 Baseline testing
- Profile of mood states
- Symbol digit modalities
- Stanford sleepiness scale
- Simple auditory RT
- Four choice RT

1100 Eat lunch

1145 Take melatonin capsules

1200 Blood sample #2
- Stanford sleepiness scale
- Wilkinson auditory vigilance

1300 blood sample #3
- Profile of mood states
- Symbol digit modalities
- Stanford sleepiness scale
- Simple auditory RT
- Dual task
- Four choice RT

1400 Blood sample #4
- Stanford sleepiness scale
- Wilkinson auditory vigilance

1500 Blood sample #5
- Profile of mood states
- Symbol digit modalities
- Stanford sleepiness scale
- Simple auditory RT
- Dual task
- Four choice RT

1600 Blood sample #6
- Profile of Mood States
- Symbol digit modalities
- Stanford sleepiness scale
- Simple auditory RT
- Four choice RT

1630 Blood sample #7
- Remove catheter
- Release subject

Task name	Approximate duration (min)	Number of repetitions
Profile of mood states	5	4
Symbol digit modalities	2	4
Stanford sleepiness scale	1	6
Simple auditory reaction time	5	4
Dual task	30	2
Four choice reaction time	5	4
Wilkinson auditory vigilance task	60	2

used to measure temperature (Model No. 403001, Becton Dickinson Consumer Products).

Subjects were required to sit at an assigned computer workstation with eyes open, and to complete interactive computer tasks throughout the day. A standard lunch was served between 1100 and 1130 hours and toilet breaks (< 5 min) were allowed. The task order and time of testing were held constant across test days (see Table 1 for detailed schedule).

Melatonin assay. Melatonin concentration measurements represent the mean of duplicate 1 ml serum samples measured by radioimmunoassay (RIA) using CIDtech Ultraspecific Melatonin Antiserum (CID-tech Research Inc., Hamilton, Ontario; Brown et al. 1983). The assay procedure is described in detail by Brzezinski et al. (1987). Briefly, the melatonin in 1-ml samples of serum were extracted into 5 ml chloroform; the organic extracts were evaporated to dryness under a stream of nitrogen; and the residues were then redissolved in phosphate buffer. Samples of the buffered serum extracts were then analyzed by RIA. The interassay coefficient of variation was 9.1% for a mean serum concentration of 102 pg/ml. The sensitivity of the assay (defined as three times the standard deviation of maximum binding) in the present study was 8 pg/ml.

Performance test battery. We selected performance tasks and mood inventories that have been shown to be sensitive to the effects of melatonin, various hypnotics, other pharmacologic agents, and sleep loss (Lieberman et al. 1984a,b, 1986, 1987; Banderet and Lieberman 1989). All performance tasks were administered on AT-class microcomputers equipped with hard drives and color VGA monitors. The computers were modified to port the speaker output to an audio amplifier (Realistic model: SA-150) equipped with stereo headphones (Realistic model: NOVA'40). To reduce the possibility of experimenter-induced bias, all tasks and instructions were automated.

Dual task. In this test, the subjects must simultaneously perform two tasks, a modified version of the Bakan vigilance test (Jones et al. 1979) and the "estimation of two classes of events in a signal stream" (PROP) test (Smith et al. 1987). The Bakan vigilance task presents, on the CRT screen, a sequence of three-digit numbers every 1.5 s for 30 min. Each successive number usually differs from the previous number by one digit. However, occasionally all three digits are repeated. The subjects' task is to detect the occurrence of these repeated sequences and to respond by pressing a key on the computer keyboard. Six blocks of 200 trials were presented during each test. Twenty-two three-digit number sequences were repeated during each block of trials. A single digit or letter is presented to the right of, and simultaneously with, the three-digit number of the Bakan series. At the end of each block of trials, the subjects were required to estimate the proportion of letters (versus numbers) occurring in the last block of PROP stimuli. Subjects responded by moving a cursor on the computer screen to select a choice. The actual proportion varied randomly between 0.2 and 0.8, in increments of 0.1, for each block of 200 stimuli. The number of true positive and true negative responses, as well as the proportions estimated and those actually presented throughout each block of trials, were retained in a computer file.

Wilkinson Auditory Vigilance task. The Wilkinson Auditory Vigilance task is a computer adaptation of the task described by R.T. Wilkinson (1969), with several modifications. Throughout the task, subjects must listen to a series of 500 Hz, 70 db (SPL) tones, presented at a rate of one tone every 2 s. The tones are of two lengths, long (500 ms) and short (variable ms). The subjects' task is to press a key when the short tone is heard. The current version of the task was similar to Wilkinson's original version in that the tones were presented for 1 h (1800 tones) and 40 (2.2%) of the tones were short. Because the subjects were tested in a quiet room using headphones, no masking noise was used. In addition, the difficulty of short tone detection was evaluated and adjusted individually for each subject during training so each subject detected a short tone approximately 50% of the time (Lieberman et al. 1987). The length of the short tones ranged from 350 to 450 ms (Mean and Mode = 400 ms). Investigators monitored the subjects throughout administration of this task to ensure that they did not sleep or close their eyes. The numbers of true positive, true negative, and premature responses were retained in a data file.

Four Choice Reaction Time (RT). This test resembles the Wilkinson Four Choice RT task and is a measure of visual vigilance (Wilkinson and Houghton 1975). Subjects are presented with a series of visual stimuli at one of four adjacent spatial locations on a CRT screen. The subject must correctly indicate the location of each stimulus by striking one of four corresponding adjacent keys on a microcomputer keyboard. Four hundred trials were administered. The mean response latency and variances, as well as the number of true positive and true negative responses, were registered and retained in a data file. The numbers of premature responses (responses made during the 400-ms pause between stimuli) and time-out errors (failure to respond within 2000 ms of stimulus presentation) were also retained. Five warm-up stimuli and the response errors were not included in the total number of trials presented.

Simple Auditory Reaction Time. In this task, the subject responds as rapidly as possible to the onset of an auditory signal. The test trials are presented in rapid succession following five warm-up trials. A 300-ms, 360-Hz tone warns the subject that a trial is about to begin. After a random delay of 100–900 ms, a 1000-Hz tone signals the subject to respond. Subjects are instructed to respond as quickly as possible after the onset of the 1000-Hz tone. The subjects' response latency appears on the computer screen for 300 ms between each trial. A warning message accompanied by an oscillating error tone occurs if the subject responds prematurely (prior to or within 50 ms of the onset of the stimulus tone) or fails to respond within 2000 ms. The subject must acknowledge premature and time-out errors by pressing the Enter key. A 1000-ms delay occurs between error acknowledgment and the next trial to allow time for the subject to reposition his hand. The response latency and variance, as well as the number of premature and time-out errors, were retained in a data file. The task continues until 200 reaction times have been recorded.

Symbol Digit Modalities task. This task is a microcomputer implementation of the paper-and-pencil symbol Digit Modalities test (Smith 1967). The task consists of a display of nine symbols in a row of boxes at the top of the screen, a display of nine corresponding numbers (i.e., 1 through 9) in a row of boxes below the symbols, a "cue" box and an "answer" box. The subject must use the numerical keypad to enter the number corresponding to the symbol that appears in the "cue" box, using the symbols and numbers at the top of the screen as a guide. The task is timed and the subject is instructed to enter as many answers as possible within 90 s. The first five symbols presented are considered practice trials and are not included in the final output or in the timed portion of the test. The symbol associated with a given digit does not change within a 90-s set of trials, but does change (randomly) each time the task is activated to prevent subjects from memorizing response keys. No response feedback is given to the subject, but a warning tone occurs if the subject presses a non–numerical key on the keyboard.

Mood and sleepiness measures. The Profile of Mood States (POMS: McNair et al. 1971) and Stanford Sleepiness Scale (SSS; Hoddes et al. 1973) were adapted to computer administration so the subjects could indicate choices by moving a cursor on the CRT screen instead of using a pencil and paper. The SSS is a self-rated 7-point scale designed to quantify the progressive stages of the sleep-alertness continuum. The POMS is a self-report scale that consists of 65 adjectives, each of which is rated on a 5-point scale. Factor analysis yields the following factors: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment.

Data analysis. Only after-treatment measures were analyzed. The dependent measures collected were each analyzed using a repeated measures, within subjects, 5 × 5 Latin Square analysis. As suggested by Winer (1972), orthogonal planned contrasts were used to test for significant differences among the melatonin/placebo treatment main effect of each analysis. The contrasts selected for the treatment effect were: 1) placebo versus 10, 20, 40, and 80 mg melatonin; 2) 10 versus 20, 40, and 80 mg melatonin; 3) 20 versus 40 and 80 mg melatonin and; 4) 40 versus 80 mg melatonin. Only treatment effects for which there are significant contrasts are reported. The treatment main effect for each of the reported contrasts is significant at the $P < 0.05$ level. The data for one subject's fifth test session were lost due to experimenter error and group means were substituted to maintain the integrity of the Latin Square for analysis purposes. Pretreatment measures are shown in the figures to illustrate baseline uniformity. The Greek symbol delta "Δ" is used to indicate "a change of".

Results

Serum melatonin levels

The mean serum melatonin levels are illustrated in Fig. 1. The mean (SEM) areas under the time-melatonin-concentration curve (AUC) between 1000 and 1640 hours for the placebo, 10, 20, 40, and 80 mg treatment conditions were 60 (1.5), 12 228 (5736.1), 27 186 (14 268.8), 52 557 (26 401.6), and 106 223 (63 038.3) pg/ml, respectively. Serum melatonin AUCs were roughly proportional to the doses administered and differed significantly among the five treatment conditions [$F(4, 60) = 42.67$, $P < 0.001$] and all planned contrasts were significant ($P < 0.01$). The order and treatment by order effects were not significant.

Oral Temperature

The mean oral temperatures measured under each treatment condition are illustrated in Fig. 2. Oral temperature changed significantly with both treatment and time [$F(4, 60) = 11.81$, $P < 0.001$; $F(4, 60) = 7.69$, $P < 0.001$, respectively]. Contrasts indicate that oral temperatures measured during the placebo treatment were an average of 0.45°F higher than those measured during the melatonin treatments [$F(5, 15) = 4.91$, $P < 0.007$]. No significant dose-related differences in oral temperature were found among the melatonin treatments.

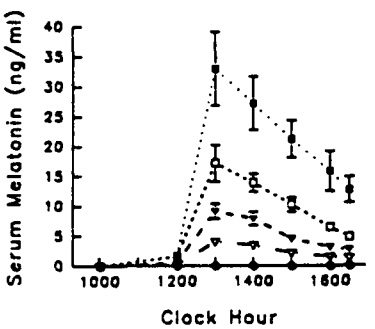

Fig. 1. Mean serum melatonin profiles of 20 subjects sampled at intervals after ingesting 0(●), 10(▽), 20(▼), 40(□), 80(■) mg of melatonin or placebo at 1145 hours

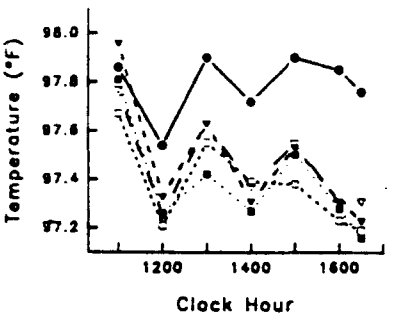

Fig. 2. Mean oral temperatures measured at intervals throughout the day after ingesting melatonin or placebo at 1145 hours ($n = 20$). (●) 0 mg; (▽) 10 mg; (▼) 20 mg; (□) 40 mg; (■) 80 mg

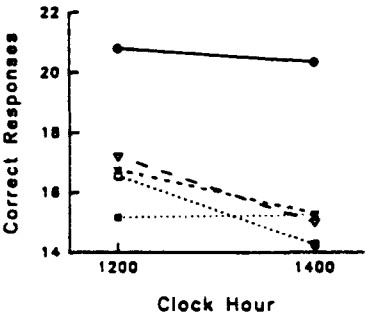

Fig. 3. Mean number of correct responses on the Wilkinson Auditory Vigilance Task after ingesting melatonin or placebo at 1145 hours ($n = 20$). (●) 0 mg; (▽) 10 mg; (▼) 20 mg; (□) 40 mg; (■) 80 mg

Treatment effects

While all of the melatonin doses administered were effective in producing performance changes, the observed changes were not proportional to the doses. The number of Wilkinson Auditory Vigilance task correct responses decreased significantly with melatonin ingestion, as illustrated in Fig. 3 [$F(4, 60) = 8.91$, $P < 0.001$]. Contrasts indicate that there were significantly fewer correct responses during melatonin treatment than during placebo treatment (Δ 4.89, $P < 0.001$). Mean Four Choice RT latencies increased significantly during melatonin treatment [$F(4, 60) = 4.19$, $P < 0.005$]. Contrasts indicate that the mean RT increased from 374.85 ms during placebo treatment to 392.81 ms during melatonin treatment [$F(5, 15) = 7.28$, $P < 0.001$].

Stanford Sleepiness Scale responses indicate that subjects were significantly more fatigued when taking melatonin, as illustrated in Fig. 4 [$F(4, 60) = 6.11$, $P < 0.001$]. Contrasts indicate that the mean response score of 2.87, measured during placebo treatment, was significantly lower than the 3.71 measured during melatonin treatment [$F(5, 15) = 3.11$, $P < 0.040$].

Subjects' POMS responses indicate that melatonin treatment (versus placebo) caused significant differences

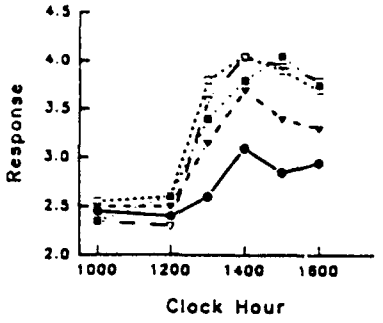

Fig. 4. Mean response scores to the Stanford Sleepiness Scale throughout testing (increased sleepiness is indicated by higher scores: $n = 20$). (●) 0 mg; (▽) 10 mg; (▼) 20 mg; (□) 40 mg; (■) 80 mg

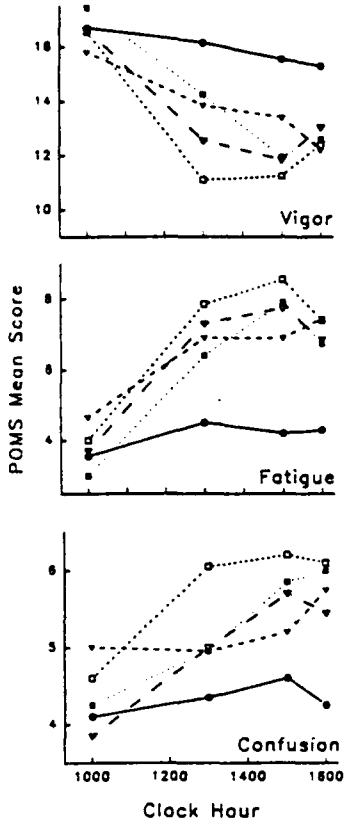

Fig. 5. Mean response scores on the Profile of Mood States questionnaire Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment scales measured throughout testing. Capsules containing melatonin or placebo were ingested at 1145 hours ($n = 20$). (●) 0 [illegible]; (▽) 10 mg; (▼) 20 mg; (□) 40 mg; (■) 80 mg

in feelings of Vigor, Fatigue, and Confusion, as illustrated in Fig. 5 [$F(4, 60) = 4.25$, $P < 0.004$; $F(4, 60) = 4.11$, $P < 0.005$; $F(4, 60) = 2.83$, $P < 0.032$, respectively]. Contrasts indicate that subjects felt more Vigorous and less Fatigued when taking placebo than when taking melatonin. Mean placebo/melatonin response differences for the subject responses on the Vigor and Fatigue scales were Δ 3.13 [$F(5, 15) = 4.60$, $P < 0.01$] and Δ 3.00 [$F(5, 15) = 5.01$, $P < 0.007$], respectively. Placebo/melatonin contrasts on the Confusion scale approached significance ($P < 0.053$).

Order effects

The number of correct and incorrect responses measured on the Dual and Wilkinson Auditory Vigilance Tasks decreased significantly on successive test days. The number Four Choice RT responses and the latency of those responses decreased significantly on successive test days. The number of correct responses measured during the Symbol Digit Modalities test increased significantly on successive test days. No significant order effects were found for the remaining measures. All treatment by order interactions were non-significant.

Discussion

These data indicate that ingestion of melatonin (10–80 mg) at 1145 hours resulted in correspondingly increased levels of circulating melatonin. All treatments, compared to placebo, significantly decreased oral temperature, feelings of Vigor, and number of correct responses on the Wilkinson Vigilance task. Melatonin ingestion also increased feelings of Sleepiness, Fatigue, and Confusion, as well as Four Choice RT response latency. The effects of melatonin ingestion, at the doses tested, are similar to those reported for sleep deprivation (Wilkinson 1968) and drugs with sedative-like properties (Hindmarch 1980; Johanson and Uhlenhuth 1980; Johnson and Chernic 1982). Results of this study suggest that the amount of melatonin necessary to induce sedative-like effects in a single administration is lower than previously reported (Lieberman et al. 1984a,b; Nickelsen et al. 1989; Waldhauser et al. 1990).

No untoward side effects or adverse reactions to exogenous melatonin were observed throughout the study. Hourly variations in oral temperature (Fig. 2) were present in the data and appeared to be consistent over test times and dates. These small ultradian fluctuations in oral temperature may be related to procedural factors such as food consumption or sedentary task performance. The decrements in performance (order effects) on the Dual, Wilkinson Auditory Vigilance, and Four Choice RT tasks have been previously observed (Dollins et al. 1993) and may be attributable to motivational factors. Task administration was menu driven and investigator comments concerning subjects' performance were specifically avoided to reduce the influence of demand characteristics. It is likely that subjects' motivation to perform at peak level was reduced by the fatiguing and unrewarding nature of these tasks on successive test days. The reported improvement in Symbol Digit Modalities task performance, a short and relatively stimulating task compared to the vigilance tasks, may be attributable to subjects increased familiarization with the input keypad (i.e., a learning effect).

Ingestion of melatonin in the 10–80 mg dose range did not elicit discernible dose-related behavioral effects. Because significant changes in oral temperature, mood, and performance measures were observed at all dose levels examined, it is likely that the biochemical mechanisms mediating melatonin's effects were saturated by the low-

est dose tested. Future studies should test the possibility that behavioral dose-response curves might be generated using lower melatonin doses (i.e., under 10 mg). The level of circulating plasma melatonin necessary to induce hypnotic symptoms, in the absence of normal nocturnal fatigue, should be determined. It is possible that any increase in circulating melatonin levels, beyond or within normal nocturnal physiological levels, will produce sedative-like effects. Melatonin may be, in effect, a naturally occurring hypnotic. Therefore, one function of melatonin in humans may be to induce sleep at night in the dark.

The observed sedative-like effect of a low pharmacologic dose of melatonin may provide grounds for reinterpretation of reported shifts in circadian entrainment in humans following administration of 0.5–5 mg melatonin (Arendt et al. 1984; Arendt and Broadway 1987; Lewy et al. 1992). It is possible that these effects were due to the direct acute hypnotic properties of melatonin rather than indirect effects on the circadian system. Such a hypothesis is consistent with the observation that traditional hypnotics speed re-entrainment (Nicholson et al. 1986; Joy et al. 1989; Van Reeth and Turek 1989)

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Report AFOSR-90-0125

Strategies to Sustain and Enhance Performance in Stressful Environments

Effects of L-Tyrosine Ingestion During Sustained Simulator Flight Following Sleep Deprivation

Andrew B. Dollins
Harris R. Lieberman
Richard J. Wurtman
Massachusetts Institute of Technology
Department of Brain and Cognitive Sciences
Building E25-615, 77 Massachusetts Ave.
Cambridge, MA 02139

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Building 410
Bolling AFB DC 20332-6448

Abstract

Animal studies indicate that administration of the large neutral amino acid tyrosine, a precursor of the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine, in pharmacologic quantities, reduces physiologic and behavioral decrements induced by highly stressful conditions. More recent investigations suggest that tyrosine administration may ameliorate decrements in human mood, performance, and physiologic tolerance induced by cold or cardiovascular stress. This study was designed to investigate the effects of tyrosine on humans required to perform a psychologically and physiologically stressful task. Fourteen healthy male pilots ingested a pharmacologic dose of tyrosine (100 mg/kg of body weight) at 0100 and 0300 h during a simulated nighttime (2400 - 0800 h) cross-country flight, preceded by 19 hours of sleep deprivation. Mood, sleepiness, reaction time, and execution accuracy of a series of flight maneuvers were measured throughout testing. Analysis indicates that error levels were smaller when ingesting tyrosine, relative to placebo, on a significant proportion (0.79, $p < 0.001$) of the flight instruments scored. The data further suggest that flight performance deteriorated between 2400 and 0400, the nadir of normal human circadian rhythms, then improved somewhat after 0600 h. A similar circadian pattern was also found in oral temperature, reaction time latencies and timeout errors, and self-reported feelings of tension.

INTRODUCTION

Tyrosine, a large neutral amino acid normally present in protein foods, is the precursor of the catecholamine (CA) neurotransmitters dopamine (DA), norepinephrine (NE), and epinephrine. When it is systematically administered in pharmacologic quantities, it can, under highly stressful conditions, increase brain CA concentration and turnover (Gibson & Wurtman, 1978; Wurtman, Larin, Mostafapour & Fernstrom, 1974; Wurtman, Hefti & Melamed, 1983). There are no known adverse effects of tyrosine administration. Because it is normally present in ordinary foods in substantial quantities and is rapidly metabolized, its administration is unlikely to have long-term toxicity or unwanted side-effects. Since it only exerts its effects when a localized deficiency state exists, the effects appear to be system-specific and only present when needed (i.e., when local CA stores are diminished). Therefore, its actions are likely to be more "specific" than most drugs, but possibly less potent. Tyrosine availability is rate-limiting for the synthesis of its neurotransmitter products in the brain only when a higher than normal level of transmitter release by catecholaminergic neurons is occurring. When CA neurons are firing frequently and, therefore, releasing more transmitter (DA or NE), they may require more of the precursor - tyrosine, the substrate for transmitter biosynthesis. Frequent neuronal firing may enhance the kinetic properties of tyrosine hydroxylase causing this rate-limiting, catecholamine-synthesizing enzyme to be more susceptible to control by this amino acid; it may also deplete the tyrosine pools within nerve terminals (Lovenberg, Bruck & Hanbauer, 1975; Milner, Reinstein & Wurtman, 1987; Weiner, Lee, Dreyer & Barnes, 1978).

A number of animal studies have demonstrated that tyrosine, given either acutely (in a single dose) or chronically in the diet, reduces adverse physiological and behavioral concomitants of acute stress. Locomotor activity, rearing, and hole-poking behavior were significantly reduced following 60 min of tail-shock stress in rats pretreated with saline but not with tyrosine (Lehnert, Reinstein, Strowbridge & Wurtman, 1984; Lehnert, Reinstein & Wurtman, 1984; Reinstein, Lehnert & Wurtman, 1984). Tyrosine pre-treatment has been found to restore normal levels of aggressive behavior in animals that have been subjected to cold-water stress (Brady, Brown & Thurmond, 1980). In a stressful behavioral procedure, , the Porsolt swim test (Porsolt, Anton, Blavet & Jalfre, 1978), sometimes considered to be a learned helplessness paradigm and used to screen drugs for anti-depressant activity, significant dose-related potentiation of escape behavior following tyrosine administration has been observed (Gibson, Deikel, Young & Binik, 1982). Specifically, animals pretreated with tyrosine (or phenylalanine which is metabolized to tyrosine) continued to swim significantly longer than placebo-treated controls. Tyrosine has also been reported to prevent decrements in performance among rats subjected to acute cold stress (Rauch &

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Lieberman, 1990). Acute administration of tyrosine can lower blood pressure (BP) in spontaneously hypertensive rats that are subjected to stressful testing conditions (Sved, Fernstrom & Wurtman, 1979) and, by acting on sympathetic instead of brain neurons, raise BP in hypotensive animals (Conlay, Maher & Wurtman, 1981; Conlay, Maher & Wurtman, 1985). Tyrosine, in a dose-dependent manner, also decreases the vulnerability of the canine heart to ventricular fibrillation and may, therefore, prevent sudden stress-induced cardiac arrest (Scott, DeSilva, Lown & Wurtman, 1981). Tyrosine may have beneficial effects on the neuroendocrine response to stress since it blocks the rise in plasma corticosterone that occurs after unavoidable stress (Lovenberg, Bruck & Hanbauer, 1975).

Studies of tyrosine administration to unstressed humans have demonstrated possible beneficial effects in the treatment of essential hypertension (Mauron, 1986) and depression (Gelenberg, Wojcik, Gibson & Wurtman, 1983). No adverse effects of such treatment were noted (Glaeser, Melamed, Growdon & Wurtman, 1979; Lieberman, Corkin, Spring, Growdon & Wurtman, 1983). However, participants in these studies were not subjected to experimental stressors and it is under stressful conditions that tyrosine would be expected to have positive effects on behavior.

We are aware of only two tyrosine studies in which human volunteers were subjected to psychologically and physiologically stressful conditions. The first employed acute exposure (4 h) to a combination of hypobaric hypoxia (corresponding to 13,800 or 15,500 ft) and cold (60^{o} F; Banderet & Lieberman, 1989). Tyrosine appeared to have robust effects among those individuals who responded most adversely to the stressors on each behavioral task. The second study employed repeated exposure to acute cardiovascular stress - lower body negative pressure (LBNP: Lieberman, Dollins & Wurtman, 1990). Effects of acute tyrosine administration (100 mg/kg) included: an overall increase in pulse pressure (LBNP typically reduces pulse pressure); an increase in auditory event related potential amplitude (P300-N300); and a statistically nonsignificant mean increase of 2.5 min of LBNP tolerance among subjects who were unable to tolerate LBNP for the full 39-min exposure. Many of the decrements in performance, mood, and symptoms induced by these treatments, including functions believed to be regulated by catecholaminergic neurons, were mitigated by tyrosine treatment. The effects of tyrosine on stress were recently reviewed by Owasoyo, Neri, and Lamberth (1992).

The current study was undertaken to examine the physiological, psychological, and behavioral effects of acute tyrosine administration on normal healthy males in a more applied circumstance - sleep deprivation combined with sustained night-time simulator flight.

METHODS

Subjects. Fourteen healthy male FAA- or USAF instrument rated pilots participated in this study (mean age = 43.8 years $\pm$ 9.4 SD). Each subject participated in two 2.0 h training sessions, to familiarize him with the battery of performance tasks, the flight simulator, and the testing procedures, as well as two 27 h test sessions (0500 - 0800 h). Subjects were paid $400.00 for participating in the study. All subjects met the medical requirements for human subjects specified by the USAF Armstrong Laboratory Advisory Committee on Human Experimentation and signed an informed consent

form (in accordance with AFR 169-3 and the MIT Committee on the Use of Humans as Experimental Subjects) prior to testing. The only physical complaints reported throughout testing were fatigue (all subjects) and headache (one subject).

Apparatus. Performance task and mood data were collected, prior to simulated flight, using a stock Zenith Z-200 microcomputer (Zenith Data Systems, St. Joseph, MI) equipped with a Gravis Mk VI joystick (Advanced Gravis Computer Technology Lts. Bellingham, WA) connected via a Magnitronic B107 game I/O card (Magnitronic, Taiwan). A Singer/Link General Aviation Trainer GAT-3 (USAF T-40, model GAT-3 A/F37A-T-40, Singer-General Precision, Inc.) was used to collect data during simulated flight. The T-40 simulates the North American Sabreliner (USAF T-39) business jet, with cockpit motion and engine sound simulation. It provides the pilot with standard aircraft cockpit instrumentation including: pitch, bank, altitude, airspeed, heading, turn rate, turn coordination, and vertical velocity. A wiring harness, developed in-house, was attached to the T-40 to measure voltages corresponding to simulator airspeed, heading, pitch, bank, glide slope, vertical velocity, altitude, turn rate, localizer deviation, and course deviation via an Analog-to-Digital converter (DAS-20, KEITHLEY Metrabyte, Taunton, MA). A 20 Mhz 80386 computer (Model CPD 320, CompuAdd, Houston, TX) equipped with two VGA monitors via a Dual VGA+ board (Colorgraphic Communications Co., Atlanta, GA) was used for data acquisition and processing. The first VGA monitor was located outside the simulator and was used to monitor system operations. The second VGA monitor, used for instructing the pilot, was positioned in the windscreen of the T-40 stimulator. The 80386 computer speaker output was ported to an audio amplifier connected to headphones (Model SA-150 and Nova-40, respectively, Radio Shack, Ft. Worth, TX) for EEG stimulus presentation.

Flight Tasks. The pilots completed repetitions of seven flight maneuvers throughout the simulated flight. The computer monitor mounted in the windscreen of the T-40 displayed instructions to the pilot. A digital display located in the lower right corner of the monitor indicated the time remaining to task completion. This display was updated every five seconds. Each flight task was composed of a one minute instruction period followed by an execution period. The pilots were instructed to maintain the current altitude, heading, and airspeed throughout the instruction period and to begin execution of the maneuver only when the one-minute instruction period was completed. They were also instructed to maintain the final altitude, heading, and airspeed when a maneuver was completed. The instructions for each task would inform the pilot which task to perform, including specific directions. The initial and final heading, airspeed, and altitude were always displayed. The desired bank and angle of turn were displayed for tasks requiring turns and the vertical velocity for tasks requiring changes in altitude.

The seven flight tasks used to assess flight performance included: level flight; straight ascent; straight descent; level turn; ascending turn; descending turn; and instrument approach. The altitude, bank, heading, air speed, and vertical velocity were recorded each second (the average of three samples per second) throughout execution of all tasks. Course deviation and glide slope were also recorded during the instrument approach.

Mood and Sleepiness Inventories. The Profile of Mood States (POMS; McNair et al. 1971) and Stanford Sleepiness Scale (SSS; Hoddes et al. 1973) were adapted to computer administration so the subjects could indicate choices by manipulating the joystick to move a cursor on the CRT screen instead of using a pencil and paper. The SSS is a self-rated 7-point scale designed to quantify the progressive stages of the sleep-alertness continuum. The POMS is a self-report scale that consists of 65 adjectives, each of which is rated on a 5-point scale. Factor analysis yields the following factors: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment. These tests were administered at 2400, 0200, 0400, and 0600.

Simple Visual Reaction Time (RT). Simple reaction time to the onset of a visual stimulus was measured during this task. The CRT displayed a 1.5 by 3.5 cm rectangle, which served as a visual fixation point throughout the task. When the task was begun, the word READY would appear in the rectangle for 300 ms and then disappear. A random delay of 100 to 3000 ms occurred, then a white bar filled the rectangle. Subjects were instructed to press a joystick response key and to respond to the appearance of the bar as quickly as possible by releasing the response key. An message would instruct the subject to resume pressing the response key as soon as a response was made. The response latency appeared (in ms) on the CRT screen immediately after each response. After a 300 ms delay the word READY would appear again and the next trial would begin. The sequence continued until one hundred trials were completed. Premature responses caused an error message to appear as did waiting more than three seconds to respond. Mean response latency and variance, as well as the number of premature and time-out errors were retained for statistical analysis.

Event Related Potentials. Cortical activity was recorded, using Grass E-5 electrodes (Grass Instruments Inc., Quincy, MA), from 10-20 system Fz and Pz positions referenced to linked mastoid processes (M1 & M2). The electrooculogram (EOG) was recorded using Grass E-5 electrodes placed above the right eye and below the left eye. Electrode impedances were checked to ensure impedances below 5 K ohms prior to each recording period. Data Inc. differential amplifiers (Model 2124, Ft. Collins, CO) were used to preamplify all signals and subsequent amplification was implemented using a Grass polygraph. Preamplifier EEG gains were set to 2 K and the low- and high-pass filters were set to 50 and 0.5 Hz respectively. The electrooculography high- and low-pass filters were set to 1000 and 0.5 Hz respectively (60 Hz notch filter active) and the gain was set to 1 K. All electrophysiologic signals were recorded on magnetic tape (Model V-Store, Racal Recorders Limited, Hythe, Southampton, England), along with a 50 uV calibration signal at the beginning of each session for subsequent off-line analysis.

Auditory ERP activity was collected using the "oddball" paradigm (Sutton, Braren, Zubin & John, 1965). Subjects were exposed to a series of frequent (2000 Hz) and infrequent (1000 Hz) stimuli, and asked to count the number of rare stimuli. The P300 wave is a large positive slow wave that occurs approximately 300 msec after the onset of the rare stimuli in the series. The latency and/or amplitude of the P300 wave vary, in a systematic manner, with a wide variety of testing circumstances (Donchin, Karis, Bashore, Coles & Gratton, 1986). A total of 660, 50 msec, stimuli were presented at the rate of 1.01 tone/sec. The infrequent tones (20%) were randomly distributed

throughout the frequent tones. Subjects were not told the correct number of infrequent tones until all testing was complete. The tones were generated by porting the speaker output from the 80386 computer to the SA-150 integrated stereo amplifier. Subjects heard the tones (90 dB SPL) over Nova-40 headphones. A 50 msec stimulus marker was recorded on magnetic tape before the onset of each stimulus, simultaneous with the electroencephalographic and electrooculographic signals.

Electrophysiologic data for each trial were sampled at a rate of 512 samples per second for 600 msec. Averaging was time-locked relative to the stimulus marker. The N100, P200, N200, P300, and N300 peaks and troughs were identified by moving a cursor along each average waveform as it was displayed on a computer monitor. The sequence of average waveforms displayed was randomized over subject and treatment condition throughout this process to prevent experimenter bias during peak and trough identification. The data for subjects with an average of fewer than 45 trials were omitted from further processing. Data for four subjects were lost due to equipment malfunction. Of the remaining 10 subjects, only six had more than 45 artifact free trials during all recording sessions of both test sessions.

Procedures. All simulator testing occurred in the high-bay area of Brooks AFB building 170. Sleep deprivation occurred in a nearby room in the same building. The study employed a double-blind placebo-control crossover design to eliminate subject and experimenter bias. Subjects participated in two 2 h training sessions to familiarize them with the T-40 simulator and testing procedures. Each subject then participated in two 27 h test sessions separated by at least fourteen days. Subjects were asked not to ingest caffeine, tobacco, and alcohol for twenty-four hours prior to testing and to fast between 2200 h and 0500 h, prior to testing. Subjects were not tested on Mondays or following holidays to reduce the effects of irregular schedules. The subjects received tyrosine or placebo (100 mg/kg in #1 capsules) in a divided dose (50 mg/kg per dose) at 0100 and 0300 h during each test session.

Subjects were transported between their homes and Brooks AFB during testing. Each subject arrived for testing at 0500 h. Between 0500 and 2400 h, subjects remained in a windowless room (ambient light approximately 300 lux) and participated in sedentary activities (reading, watching television, listening to the radio). Vital signs, including heart rate, blood pressure, and oral temperature were measured and recorded every two hours between 0500 and 2300 h and between 2400 and 0800 h. Urine samples were collected during each micturition. Subjects also completed a battery of computer-interactive performance tasks, requiring approximately 35 minutes to complete, every two hours between 0500 and 2300 h. Data collected during these tasks will not be reported in this manuscript. Subjects were given breakfast (a choice of cereal bars or breakfast tacos - a local specialty) at 0730 h, lunch (a hamburger or submarine sandwich) at 1330 h, dinner (pizza, steak, or salad) at 1730 h, and a snack of cereal bars at 2130 h. They were allowed free access to non-caffeinated beverages (water, orange juice, root beer, ginger ale) throughout testing. At 2330 h, subjects were escorted to the T-40 simulator and EEG electrodes were attached.

The experimenter monitoring the subjects' performance and the subject communicated via an intercom system using standard headsets throughout the

flight. Planned communication was limited to: 1) routine requests by the experimenter for the simulator's current altitude, heading, and airspeed (during the last minute of every third task); 2) informing the pilot that the simulator position was about to be reset prior to the instrument approach task; 3) informing the pilot that he had exceeded designated target flight parameters by 90^{o} or 1000 ft (four occasions); 4) inquiring if the pilot had any beverage or snack requests for the next break; and 5) informing the pilot that the experimenter was temporarily leaving his station. Unplanned communication occurred when there was a malfunction in the simulator (three occasions); the pilot informed the experimenter that an electrode had detached; the pilot requested an unplanned break for micturition (one pilot required hourly breaks throughout both sessions); or the pilot asked the time remaining until the next break. While such communication was discourages, the pilots would occasionally attempt casual conversation with the monitor and/or sing to themselves.

Simulator parameters were pre-set to the same specifications prior to each simulated flight. All 12 and 24 h timepieces visible to the pilot were removed or disabled during flight. All verbal references to time were avoided and/or made relative to the current time (i.e., 'three more maneuvers until the next break', 'two more flight legs tonight', 'you're about half way through').

The simulated flight was divided into four flight legs lasting approximately two hours each. The maneuvers included in each flight leg are listed in Tables 1 through 4. The simulator's position was reset to a pre-determined heading, altitude, and airspeed 20 seconds prior to the execution instruction of the instrument approach task. All flight maneuvers, except the instrument approach, ended at the heading, altitude, and airspeed required to begin the next maneuver. All subjects flew the same course during all test sessions. All flight instructions were presented on the computer monitor mounted in the T-40 windscreen. The simulator's current position was frozen (activation of a switch on the operator's console maintained the simulator's current airspeed, heading, and altitude until the switch was deactivated) at the beginning of each flight leg while the subjects vital signs were recorded and POMS, SSS, and Simple RT tasks were completed. Evoked Potentials were collected at 2400, 0400, and 0600 h. Subjects were allowed to exit the simulator for micturition between flight legs. The simulator's position was also frozen when the treatment capsules were administered at 0100 and 0300 h.

Data Reduction. Similar flight maneuvers were normalized mathematically. Left turns were converted to right turns by adding appropriate offsets to the original heading and bank values. Initial altitudes for each maneuver were, again, normalized by adding appropriate offset values to the original recorded altitudes. For example, all level flight tasks were mathematically converted to a heading of 360 degrees and an altitude of 2500 ft, regardless of the original heading and altitude. As seen in Table 5, between three and eight repetitions of each of the thirteen tasks resulted from this manipulation.

Deviation from an ideal criteria was evaluated for each of the following flight measures: altitude, bank, heading, air speed, and vertical velocity (glide slope and course deviation during the instrument approach were also evaluated). An evaluation criteria template was designed for each flight

measure evaluated. Ideal templates were used for measures which should have remained constant throughout the flight (such as altitude, bank, heading, air speed, and vertical velocity during level flight). Estimated templates were calculated for measures which were expected to change during the flight maneuver (such as altitude, bank, heading, and vertical velocity during an ascending turn). Estimated templates were calculated by time locked averaging of all data collected for that flight measure during the maneuver.

A unique template was designed for the instrument approach task. The pilot flew a "missed approach" (i.e., did not actually "land" the simulator, but accelerated and climbed to a predetermined altitude and heading after descending to a altitude of 100 ft above the landing site) during the first two instrument approaches and landed during the third. Thus only the first portion of the approach could be scored. Successful completion was also dependent on an external signal (i.e., the ILS) in addition to pilot skill. The pilot did not intercept the ILS signal until he was eight miles from the landing site. The Glide Slope and Course Deviation indicators were not accurate until the ILS signal was intercepted. The following convention was adopted when scoring Glide Slope and Course Deviation performance on the first portion of the flight. Time-locked plots of Altitude, Glide Slope and Course Deviation were examined visually. The beginning of Glide Slope and Course Deviation data was defined as the point where the aircraft altitude remained below 2400 ft for a least 30 sec. Data from this point until the simulator altitude indicated that the simulator was below 500 ft or was climbing were retained for RMS analysis. The investigator was blind to treatment conditions when examining these plots.

Each template was a series of values (a line) representing the ideal instrument reading throughout the flight maneuver. The actual data collected during each flight maneuver were subtracted from its corresponding template. Thus, a perfect flight maneuver should have resulted in a straight line, indicating no deviation from the template criteria.

The data for each flight measure were then converted to offsets from the ideal using a "+"/"-" convention. For example, a heading error of 15^{o} to the left on the level flight task was converted to a heading of -15^{o} instead of a heading of 345^{o}. Values within the deviation criteria allowed by the USAF ATC Phase Training Standard for undergraduate pilot training on the USAF T-37 aircraft (ATC PTS P-V4A-A-2) were then set to zero. The ranges specified were: altitude, $\pm$ 150 ft; bank, $\pm$ 5^{o}; heading $\pm$ 5^{o}; and air speed, $\pm$ 10 knots indicated air speed. (A conservative value of $\pm$ 500 ft was chosen for vertical velocity because ATC PTS P-V4A-A-2 specifies no evaluation range for this measure.) Thus, only deviations outside of this conservative range were scored as errors. Root Mean Square (RMS) values were then calculated for the flight measures obtained from each pilot for subsequent analysis.

Missing Data. There were some missing flight performance data because two subjects chose to abort testing early on one or both nights. Some data were also missing due to simulator instrument malfunction and investigator error. Sub-group means were substituted for these missing data points. For instance, subject 06 did not complete the level flight task at 0600 h. The average 0600 h RMS value for that task, instrument, and treatment condition was substituted for that missing data point.

Data Analysis. The RMS values of each flight measure and maneuver were analyzed separately using a between-groups repeated-measures Analysis of Variance (ANOVA) resulting in a total of 67 analyses. The analysis implemented was a 2(treatment order - between) x 2(treatments tyrosine/placebo - within) x 3 to 8(repetition of task / time - within) factorial ANOVA. Repeated measure t-tests were calculated to evaluate tyrosine/placebo treatment effects during each flight maneuver repetition. A post-hoc non-parametric test was also calculated to evaluate overall effects of tyrosine/placebo treatment on flight performance. Between-groups repeated-measures ANOVAs were used to evaluate changes in: oral temperature; systolic and diastolic blood pressures; heart rate; the POMS Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment scales; the Stanford Sleepiness Scale; Simple RT latency, response latency variance, and number of premature response and timeout errors; and Auditory Evoked Potential rare trial component amplitudes and latencies. Only the results of statistically significant ($p < 0.05$) analyses are reported. Main effects and interactions which are not mentioned were not statistically significant. The Greek symbol delta "$\triangle$" is used to indicate "a change of".

RESULTS

Treatment Effects. Tyrosine treatment significantly reduced RMS errors recorded in bank [$\triangle = 1.05$, $F(1,12) = 5.166$, $p < 0.05$] and heading [$\triangle = 0.81$, $F(1,12) = 6.23$, $p < 0.05$] during straight 2000 ft descent task and in bank [$\triangle = 0.73$, $F(1,12) = 4.46$, $p < 0.05$] during the ascending 180° turn task, relative to placebo. Significant Treatment by Time interaction effects were found among: the heading measures [$F(3,36) = 3.408$, $p < 0.05$] during the straight 2000 ft descent; the bank [$F(3,36) = 4.149$, $p < 0.01$] and heading [$F(3,36) = 3.527$, $p < 0.05$] measures during the level 180° turn; and the vertical velocity [$F(3,36) = 3.163$, $p < 0.05$] measures during the flat 360° turn. The straight 2000 ft descent heading interaction indicates that RMS error was smaller when subjects ingested tyrosine, relative to placebo, as testing progressed between 2400 and 0800 h. The level 180° turn bank and heading interactions indicate that performance errors were smaller when taking tyrosine, relative to placebo, at all times except 0400 h. Flat 360° turn vertical velocity errors increased more when subjects ingested tyrosine, relative to placebo, as testing progressed between 0200 and 0800 h. Since it is possible that the three significant treatment effects may have occurred due to chance alone, given the relatively large (67) number of analysis calculated. A post-hoc analysis was calculated to determine whether errors were greater when ingesting tyrosine or placebo. The average RMS errors were greater during placebo treatment on 53 or the 67 flight performance measures. A non-parametric analysis of proportions (Bruning & Kintz, 1987) indicates that this proportion (0.79) is significantly different from the expected proportion of 0.500 ($p < 0.0001$).

Treatment Order Effects. There were no significant differences attributable to treatment order on any measure.

Time Effects. Significant changes ($p < 0.05$) over time were found in oral temperature, mood, sleepiness, reaction time, and one or more measure during every flight maneuver. These results are summarized in Tables 7 through 11.

Event Related Potentials. No significant differences in peak amplitude or latency were found among the data sampled from six subjects.

DISCUSSION

These data indicate that error levels were smaller when ingesting tyrosine, relative to placebo, on a significant proportion (0.79, $p < 0.001$) of the flight instruments scored. Significantly smaller RMS errors were found in heading and bank control during the straight 2000' descent task and in bank control during the ascending 180° turn when the pilots ingested tyrosine, relative to placebo. These data indicate that tyrosine ingestion does reduce performance decrements during sustained night-time flight preceded by sleep deprivation.

The relatively small reduction in performance decrement may be due to several factors. The effects of tyrosine, while quite specific, may be less potent than those of less specific but more powerful substances. The observed reduction in flight maneuver error following tyrosine ingestion, relative to placebo, may represent the maximum effect of tyrosine ingestion. The physiologic and psychologic effects of a simulated night-time cross-country flight may not have been sufficiently stressful. Subject comments that "this is like flying, but I need the roar of engines and smell of jet fuel to make it real" would support this hypothesis. If so, catecholamine neurotransmitter depletion may not have been sufficient for the effects of tyrosine availability to become apparent. It is also possible that mechanisms other than catecholamine neurotransmitter depletion (i.e., fatigue, sleepiness, circadian behavior rhythms) were responsible for a large portion of the performance decrement.

A second interesting result of this investigation is the demonstration that the current paradigm may be useful in testing fatigue and circadian performance. Significant time effects indicating increased self reported feelings of Tension, Depression, Fatigue, and Confusion; decreased feelings of Vigor; and decrements in performance reflected in both reaction time and flight performance accuracy measures (Tables 7 - 11) were evident between 0200 and 0600 h. These results suggest that the current paradigm may be a useful model for testing other treatments which are expected to influence sustained performance.

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Table 1

Flight Leg 1

Flight Maneuver	Final Heading[a]	Final Altitude[b]	Duration[c]
Takeoff	360	2500	6
Instrument Approach	360	2500	11
Level +180° turn	180	2500	4
Ascending -270° turn	270	5500	7
Descending +180° turn	90	3500	6
Straight Ascent	90	5500	6
Level -360° Turn	90	5500	6
Straight Descent	90	4500	5
Level Flight[d]	90	4500	11
Ascending +180° turn	270	6500	6
Descending -270° turn	360	3500	7
Straight Ascent	360	4500	5
Straight Descent	360	2500	6
Ascending +270° turn	270	5500	7
Descending -270° turn	360	2500	7

NOTE. Flight parameter instructions were as follows: 1000 ft per min ascent/descent rate; +/- 30° bank on turns; and 240 Knots Indicated Airspeed for all tasks except the Instrument Approach

[a] degrees
[b] feet
[c] minutes - including one minute instruction time
[d] Rough Air at 50% of maximum was applied between minutes 5 and 8 of the level flight task

Table 2

Flight Leg 2

Flight Maneuver	Final Heading[a]	Final Altitude[b]	Duration[c]
Ascending -180° turn	180	4500	6
Straight Ascent	180	6500	6
Level +270° turn	90	6500	5
Straight Descent	90	5500	5
Descending -270° turn	180	2500	7
Level Flight[d]	180	2500	11
Ascending +270° turn	90	5500	7
Level -180° turn	270	5500	4
Straight Ascent	270	6500	5
Descending +180° turn	90	4500	6
Level -360° turn	90	4500	6
Level +270° turn	360	4500	5
Straight Descent	360	2500	6
Ascending -270° turn	90	5500	7
Descending +270° turn	360	2500	7

NOTE. Flight parameter instructions were as follows: 1000 ft per min ascent/descent rate; +/- 30° bank on turns; and 240 Knots Indicated Airspeed for all tasks except the Instrument Approach

[a] degrees
[b] feet
[c] minutes - including one minute instruction time
[d] Rough Air at 50% of maximum was applied between minutes 5 and 8 of the level flight task

Table 3

Flight Leg 3

Flight Maneuver	Final Heading[a]	Final Altitude[b]	Duration[c]
Instrument Approach	360	2500	11
Level +270° turn	270	2500	5
Ascending -180° turn	90	4500	6
Straight Ascent	90	6500	6
Descending +270° turn	360	3500	7
Straight Descent	360	2500	5
Level -180° turn	180	2500	4
Ascending +270° turn	90	5500	7
Level flight	90	5500	11
Descending -180° turn	270	3500	6
Straight Ascent	270	4500	5
Level +360° turn	270	4500	6
Straight Descent	270	2500	6
Ascending -270° turn	360	5500	7
Descending +270° turn	270	2500	7
Level -270° turn	360	2500	5

NOTE. Flight parameter instructions were as follows: 1000 ft per min ascent/descent rate; +/- 30° bank on turns; and 240 Knots Indicated Airspeed for all tasks except the Instrument Approach

[a] degrees
[b] feet
[c] minutes - including one minute instruction time
[d] Rough Air at 50% of maximum was applied between minutes 5 and 8 of the level flight task

Table 4

Flight Leg 4

Flight Maneuver	Final Heading[a]	Final Altitude[b]	Duration[c]
Level -270° turn	90	2500	5
Straight Ascent	90	4500	6
Ascending +180° turn	270	6500	6
Straight Descent	270	5500	5
Descending -270° turn	360	2500	7
Level +180° turn	180	2500	4
Level Flight[d]	180	2500	11
Ascending -270° turn	270	5500	7
Straight Ascent	270	6500	5
Level +270° turn	180	6500	5
Descending -180° turn	360	4500	6
Level +360° turn	360	4500	6
Straight descent	360	2500	6
Ascending -270° turn	90	5500	7
Descending +270° turn	360	2500	7
Instrument Approach	360	00	11

NOTE. Flight parameter instructions were as follows: 1000 ft per min ascent/descent rate; +/- 30° bank on turns; and 240 Knots Indicated Airspeed for all tasks except the Instrument Approach

[a] degrees
[b] feet
[c] minutes - including one minute instruction time
[d] Rough Air at 50% of maximum was applied between minutes 5 and 8 of the level flight task

Table 5

Number of Flight Maneuver Repetitions

	Flight Leg				
	1	2	3	4	TOTAL
Instrument Approach	1	0	1	1	3
Level Flight (10 min)	1	1	1	1	4
Straight 1000' Ascent	1	1	1	1	4
Straight 2000' Ascent	1	1	1	1	4
Straight 1000' Descent	1	1	1	1	4
Straight 2000' Descent	1	1	1	1	4
Level 180° turn	1	1	1	1	4
Level 270° turn	0	2	2	2	6
Level 360° turn	1	1	1	1	4
Ascending 180° turn	1	1	1	1	4
Ascending 270° turn	2	2	2	2	8
Descending 180° turn	1	1	1	1	4
Descending 270° turn	2	2	2	2	8
TOTAL	14	15	16	16	61

Table 6

Summary of Flight Measures Evaluated Using Estimated Template Criteria

	Method of Template Design				
	Altitude	Bank	Heading	Air Speed	Vertical Velocity
Instrument Approach[a]	Est			Est	Est
Level Flight (10 min)					
Straight 1000' Ascent	Est				Est
Straight 2000' Ascent	Est				Est
Straight 1000' Descent	Est				Est
Straight 2000' Descent	Est				Est
Level 180° turn		Est	Est		
Level 270° turn		Est	Est		
Level 360° turn		Est	Est		
Ascending 180° turn	Est	Est	Est		Est
Ascending 270° turn	Est	Est	Est		Est
Descending 180° turn	Est	Est	Est		Est
Descending 270° turn	Est	Est	Est		Est

NOTE. Est indicates that criteria used were means of values actually measured during testing.

[a] See text for explanation of Instrument Apporach Glide Slope and Course Deviation criteria.

Table 7

Average Change in Oral Temperature, Mood, and Reaction Time throughout Testing

Measure	Flight Leg 1	2	3	4
Oral Temperature	97.498	97.132	96.961	97.058 [c]
Profile of Mood States				
Tension-Anxiety	4.822	5.643	8.000	7.572 [c]
Depression-Dejection	1.357	2.215	4.250	5.357 [c]
Anger-Hostility	1.572	2.393	2.715	3.357
Vigor-Activity	14.536	10.464	7.608	7.214 [c]
Fatigue-Inertia	5.822	10.286	15.215	16.572 [c]
Confusion-Bewilderment	4.822	6.929	10.071	10.072 [c]
Stanford Sleepiness	2.464	4.000	4.608	5.000 [c]
Simple Reaction Time				
Latency (ms)	270.436	318.736	348.212	329.084 [c]
Response Variance	13928.619	30560.595	44847.364	36141.515 [c]
Premature Responses	4.036	5.000	5.858	7.357 [b]
TimeOut Errors	0.000	0.857	2.143	1.679 [b]

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.005$

Table 8

Average RMS Error over Time During Straight Flight Maneuvers

Measure	Flight Leg 1	2	3	4	
Straight 10 min Flight					
Altitude	9.418	27.862	55.750	29.863	a
Bank	1.572	2.763	4.663	4.227	c
Heading	1.004	2.381	3.695	2.753	
Air Speed	0.939	2.101	2.681	2.112	c
Vertical Velocity	102.654	166.439	245.853	202.791	c
Straight 1000' Ascent					
Altitude	46.508	41.213	53.635	41.050	
Bank	2.315	2.641	3.959	3.399	c
Heading	1.153	1.634	2.045	1.851	
Air Speed	2.656	1.415	3.993	1.914	c
Vertical Velocity	176.533	219.183	243.036	199.575	c
Straight 2000' Ascent					
Altitude	10.622	35.027	73.971	44.293	a
Bank	1.358	3.438	4.142	3.178	
Heading	0.575	2.108	3.567	1.773	a
Air Speed	0.964	1.407	3.065	3.103	a
Vertical Velocity	85.801	140.968	217.675	160.486	c
Straight 1000' Descent					
Altitude	17.569	37.624	43.871	48.850	
Bank	1.435	2.484	3.882	3.772	a
Heading	0.698	1.272	2.585	1.457	b
Air Speed	0.801	2.014	2.622	2.769	
Vertical Velocity	86.285	168.899	201.189	208.866	b
Straight 2000' Descent					
Air Speed	29.033	29.910	50.372	26.188	
Bank	2.435	3.362	3.376	3.468	
Heading	1.266	2.485	1.714	1.726	
Air Speed	12.483	32.742	36.406	22.853	
Vertical Velocity	112.320	223.163	219.072	188.895	c

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.005$

Table 9

Average RMS Error over Time During Flat Turn Maneuvers

Measure	Flight Leg 1	2	3	4	
Level 180° turn					
Altitude	2.406	22.189	51.645	36.132	a
Bank	2.668	4.799	5.522	4.990	c
Heading	3.339	4.585	6.545	7.186	
Air Speed	0.533	1.911	3.505	1.923	
Vertical Velocity	64.657	195.461	244.587	192.555	c
Level 270° turn					
Altitude		19.505	38.319	36.293	
Bank		4.995	5.283	5.962	
Heading		8.492	7.955	9.976	
Air Speed		1.615	2.798	3.495	a
Vertical Velocity		180.492	227.488	209.573	a
Level 360° turn					
Altitude	6.126	25.956	68.337	32.260	c
Bank	2.977	5.093	5.935	4.781	b
Heading	5.080	12.578	10.494	7.515	
Air Speed	0.763	2.582	4.568	3.625	c
Vertical Velocity	93.337	227.827	302.043	203.647	c

Note. Values for the level 270° turn are averages of two repetitions.

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.005$

Table 10

Average RMS Error over Time During Ascending/Descending Turn Maneuvers

Measure	Flight Leg 1	2	3	4	
Ascending 180° turn					
Altitude	23.406	18.488	68.070	54.268	c
Bank	3.030	3.236	4.673	4.976	b
Heading	3.829	4.240	5.338	5.868	
Air Speed	1.235	1.869	4.651	4.307	a
Vertical Velocity	114.232	113.922	245.237	203.906	c
Ascending 270° turn					
Altitude	48.925	98.687	108.969	108.909	
Bank	3.175	4.795	5.672	5.201	c
Heading	6.678	8.825	7.853	8.414	
Air Speed	2.142	4.476	4.668	5.111	b
Vertical Velocity	125.804	244.529	278.287	231.238	c
Descending 180° turn					
Altitude	13.754	92.272	113.747	64.947	a
Bank	2.928	6.036	5.830	4.999	a
Heading	4.898	11.095	9.522	6.833	
Air Speed	0.924	2.963	4.170	3.032	b
Vertical Velocity	76.154	243.769	284.005	210.674	c
Descending 270° turn					
Altitude	61.12	83.050	168.721	98.029	c
Bank	3.4625	4.637	5.721	4.775	b
Heading	5.225	7.136	10.9165	6.929	
Air Speed	1.9135	3.479	4.541	3.110	c
Vertical Velocity	143.297	216.732	268.309	214.328	c

Note. Values for the 270° turns are averages of two repetitions.

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.005$

Table 11

Average RMS Error over Time During Instrument Approach Maneuvers

Measure	Flight Leg 1	2	3	4
Altitude	103.950		103.494	94.122
Bank	1.762		3.400	3.956 [c]
Heading	1.658		3.552	4.711 [a]
Air Speed	8.629		8.795	7.238
Vertical Velocity	97.551		193.406	117.677 [c]
Course Deviation	1.025		1.207	1.426
Glide Slope	2.001		2.019	2.279

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.005$

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EFFECT OF INDUCING NOCTURNAL SERUM MELATONIN CONCENTRATIONS IN DAYTIME ON SLEEP, MOOD, BODY TEMPERATURE, AND PERFORMANCE

Andrew B. Dollins*, Irina V. Zhdanova, Richard J. Wurtman,

Harry J. Lynch, and Mei H. Deng

Department of Brain and Cognitive Sciences

Massachusetts Institute of Technology

Cambridge, MA 02139 USA

To whom reprint requests should be addressed:
Richard J. Wurtman, M.D.
Massachusetts Institute of Technology/E25-604
Cambridge, MA 02139 USA
TEL: (617) 253-6732
FAX: (617) 258-6882

*Present address:

DoD Polygraph Institute
Building 3195
Ft. McClellan, AL 36205

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ABSTRACT

We examined effects of very low doses of melatonin (0.1-10 mg p.o.) or placebo, administered at 1145 h, on sleep latency and duration; mood; performance; oral temperature; and changes in serum melatonin levels in 20 healthy male volunteers. A repeated-measure double-blind Latin square design was used. Subjects completed a battery of tests designed to assess mood and performance between 0930 and 1730 h. The sedative-like effects of melatonin were assessed by a simple sleep test: at 1330 h subjects were asked to hold a positive pressure switch in each hand and to relax with eyes closed while reclining in a quiet darkened room. Latency and duration of switch release, indicators of sleep, were measured. Areas under the time-melatonin concentration curve varied in proportion to the different melatonin doses ingested, and the 0.1 and 0.3 mg doses generated peak serum melatonin levels which were within the normal range of nocturnal melatonin levels in untreated people. All melatonin doses tested significantly increased sleep duration, as well as self-reported sleepiness and fatigue, relative to placebo. Moreover, all of the doses significantly decreased sleep onset latency, oral temperature, and the number of correct responses on the Wilkinson Auditory Vigilance task. These data indicate that orally-administered melatonin can be a highly potent hypnotic agent; they also suggest that the physiological increase in serum melatonin levels which occurs around 2100 h daily may constitute a signal initiating normal sleep onset.

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INTRODUCTION

Serum melatonin levels in normal humans are very low during most of the day, but rise significantly to a mean of 45 pg/ml (range 0 to 200) between 0200 and 0400 h (1 pg/ml = 4.31 pmol/L), and remain elevated during the normal hours of sleep, falling sharply to daytime values around 0900 h (1). The physiological significance of the nocturnal increase in serum melatonin could derive from acute effects of the hormone [e.g., its ability to reduce core body temperature (2); alter thermoregulation (3); modify brain levels of monoamine neurotransmitter (4); stimulate prolactin secretion (5); or induce sleepiness (6)].

Alternatively, the nocturnal rise in serum melatonin could constitute a time signal, affecting the temporal characteristics of other circadian rhythms. Oscillations in the concentration of circulating melatonin could directly affect circadian rhythms (7-9), or could provide humoral communication of information about environmental lighting (and thus about time of day) which entrains endogenous physiological and behavioral rhythms (e.g., those associated with photoperiodism, or seasonality; 10). Some studies also suggest a role for melatonin in human development. The decrease in amplitude of the melatonin rhythm which occurs late in the first decade of life has been proposed as a factor contributing to pubescence (11), while further decrease, which occurs after the sixth decade, may contribute to disruptions in circadian rhythmicity reported by the elderly (12).

The acute effects of exogenous melatonin on human behavior have been studied only sporadically and have utilized melatonin doses which raise serum melatonin levels well beyond their normal nocturnal range. Thus, Lieberman et al. (13) found that a dose (240 mg over a 2-h period) which raised serum melatonin levels several thousand-fold impaired mood and performance. More recently, we found similar behavioral changes after a considerably lower dose of the hormone (10 mg), which still raised serum levels to approximately 40-50 times the normal nocturnal level (i.e., 4072 pg/ml; 14).

The present study was designed to determine whether much lower daytime doses, which elevate serum melatonin levels significantly but keep these levels within the normal nocturnal range, are also sufficient to produce short-term behavioral effects. If so, this would suggest a similar role for the normal nocturnal increase in serum melatonin levels. We gave the melatonin at midday (nine or more hours before the nocturnal rise) and measured mood, performance, sleepiness, and - indirectly - sleep onset.

METHODS AND MATERIALS

Twenty healthy male volunteers (Mean Age = 23.05 $\pm$ 4.22 SEM; Range 18-24 years) participated. Prior to admission to the study, each subject gave his informed consent, had a physical examination to ensure that he was in good health, and completed two 1.5-h training sessions to become familiar with testing procedures and the performance test battery. Subjects were also screened for depressive symptoms using the Hamilton Psychiatric Rating Scale for Depression (15) with a special addendum for Seasonal Affective Disorder (16); any with a history or findings of depression were excluded. All subjects were paid for their participation in the experiment.

The study was double-blind and placebo-controlled. A repeated measures, within subjects, 5x5 Latin Square design was employed. The subjects participated in five 8-h (0930 - 1730 h) testing sessions. At least five days elapsed between successive test sessions. Capsules containing 0.1, 0.3, 1.0, or 10 mg of melatonin or placebo were administered (p.o.) at 1145 h each test day. Treatment order was determined by the balanced Latin Square design.

Oral temperatures were measured hourly and blood was sampled at regular intervals via an indwelling venous catheter for subsequent serum melatonin measurement. Serum samples were separated by centrifugation and stored at -20°C until they could be assayed by radioimmunoassay for melatonin (17).

Throughout test sessions, subjects were required to sit at an assigned computer workstation with eyes open. The task order and time of testing were held constant across test days. All instructions, performance tasks, and mood questionnaires were automated to reduce the possibility of experimenter-induced bias. The performance tasks used were coded in-house and included measures of: 1) Auditory Vigilance (18); 2) Four Choice Reaction Time (19); 3) Simple Reaction Time; and 4) Symbol Digit (Modalities) Substitution (20). Mood questionnaires included the Profile of Mood States (POMS; 21) and the Stanford Sleepiness Scale (SSS; 22). Details of the tasks and their administration are published elsewhere (14). The mood questionnaires were completed at 1030 h and at hourly intervals beginning at 1200 h. The Simple and Four Choice RT and Symbol Digit Substitution tasks were completed at 1030, 1300, 1500, 1600, and 1700 h. The Auditory Vigilance task was administered at 1200 and 1400 h. Subjects were allowed to leave their workstations during lunch (a standard lunch was served between 1100 and 1130 h), toilet breaks (<5 min), and during the half hour sleep test.

Subjects participated in a sleep test between 1330 and 1400 h. They were asked to recline (on either a bed or reclining chair) and relax with their eyes closed in a quiet, darkened room. In each hand they held a 1" plastic tube which bore a positive pressure switch. They were asked to rest their hands, palm up, alongside their body and to depress the switches with the last segment of their index fingers. Release of the switch on either tube was recorded as a pen deflection on an event-recorder. An investigator remained in attendance with the subjects to ensure that they followed instructions. An event-timer solenoid was randomly activated to reduce the possibility that the soft click of the switch would be misconstrued by the subjects as a significant event. After 30 min, the subjects were asked to stop relaxing and/or were awakened. They were then asked: 1) if they fell asleep; 2) if so, how long did it take to fall asleep; and 3) to respond to the Stanford Sleepiness Scale. Latency to switch release was measured from the beginning of instruction presentation to the first full minute of switch release. Total switch release time was measured as the total length of time a recording pen was deflected (the smallest interval of pen deflection measured was 1 min, accuracy = 0.25 min). One subject failed to release a switch during a sleep test session, but was identified as asleep by his prolonged snoring. Sleep onset for this subject was recorded as occurring after two min of continuous snoring. When questioned later, the subject reported that he had indeed fallen asleep.

The after-treatment dependent measures were each assessed using a repeated measures, within subjects, 5x5 Latin Square analysis. Orthogonal planned comparisons (23) were used to evaluate differences among the melatonin/placebo treatment conditions when a significant ($p<0.05$) treatment effect was found. The comparisons chosen were: 1) placebo vs. all melatonin treatments; 2) 0.1 vs. 0.3, 1.0, and 10 mg of melatonin; 3) 0.3 vs. 1.0 and 10 mg of melatonin; and 4) 1.0 vs. 10 mg of melatonin. Pairwise comparisons were subsequently calculated for these measures (repeated measures t-tests for the Sleep Test and Melatonin data and ANOVAs for the other measures) because inspection of the data suggested that the planned comparisons provided an insufficient basis for interpreting results. Only main effects which resulted in significant contrasts, and interaction effects are reported. There were some missing data due to difficulties with equipment. Group means were therefore substituted for the 1700 h 0.1 mg, 1300 h 1.0 mg, 1700 h 1.0 mg, and 1000 h 10 mg measures for subjects 15, 03, 16, and 15 respectively, on most performance measures. Blood samples were not drawn during subject 01's placebo testing due to difficulties with catheterization and group means were substituted for this data. Group means of serum melatonin levels were also substituted for six other missing data points. The Greek symbol delta "$\triangle$" is used to indicate "an average change of".

RESULTS

Serum Melatonin Levels: Mean serum melatonin levels are illustrated in Fig. 1. The mean (SEM) areas under the time-melatonin-concentration curve (AUC) between 1000 and 1730 h for the placebo, 0.1, 0.3, 1.0, and 10 mg treatment conditions were 87.7 (5.11), 213.2 (25.02), 459.9 (62.7), 1599.0 (141.7), and 21000.4 (3752.3), respectively. Serum melatonin AUC differed significantly among the five treatment conditions [$F(4,60)=34.34$, $p<0.001$] and all planned contrasts were significant ($p<0.001$). All pairwise comparisons were also significant ($p<0.001$). The order and treatment-by-order effects were not significant.

Melatonin Treatment Effects: Significant melatonin treatment effects were found for: oral temperature [$F(4,60)=7.90$, $p<0.001$]; sleep test sleep onset latency [$F(4,60)=6.65$, $p<0.001$], sleep duration [$F(4,60)=7.80$, $p<0.001$], self-reported sleep onset latency [$F(4,60)=10.52$, $p<0.001$], and post-sleep-test SSS responses [$F(4,60)=3.11$, $p<0.05$] (Fig.2); POMS Vigor-Activity [$F(4,60)=4.16$, $p<0.01$] and Fatigue-Inertia [$F(4,60)=3.05$, $p<0.05$] (Fig.3) responses; SSS responses [$F(4,60)=2.79$, $p<0.05$]; number of correct responses on the Wilkinson Vigilance task [$F(4,60)=3.42$, $p<0.05$]; and Four Choice RT response latency [$F(4,60)=3.84$, $p<0.01$]. Table 1 summarizes the planned comparison results and Table 2 contains the mean (SEM) levels measured. The treatment-by-order interaction effects were non-significant for all measures except the SSS [$F(4,60)=4.68$, $p<0.001$]. There were no significant differences among the baseline (1000 h) oral temperature, SSS, or POMS measures. An order effect was found among the Four Choice RT response latency baseline measures [$F(4,60)=15.13$, $p<0.001$], but the treatment and treatment-by-order interaction effects were non-significant.

As Table 2 indicates, response levels for some measures did not consistently increase or decrease relative to serum melatonin levels. For example, self-reported SSS responses were greatest and POMS Vigor-Activity scale responses were smallest after ingesting 0.3 mg, rather than higher doses, of melatonin.

Pairwise comparisons were calculated to aid in interpretation of these data. Mean oral temperature measures were significantly less, relative to placebo, after ingesting 1.0 and 10 mg of melatonin (△ -0.24 and -0.37 °F, respectively. Oral temperatures measured after ingesting 1.0 (△ -0.16) and 10 (△ -0.29) mg of melatonin were also less than those measured after ingestion of 0.1 mg of melatonin. Ingestion of 10 mg of melatonin also decreased oral temperature relative to the 0.3 and 1.0 doses (△ -0.21 and -0.13, respectively). SSS responses indicated greater feelings of sleepiness, relative to placebo, after ingesting 0.3, 1.0, and 10 mg of melatonin (△ +0.51, +0.47, and +0.46, respectively). POMS responses showed a decrease in self-reported feelings of Vigor-Activity, relative to placebo, after ingesting 0.3, 1.0, and 10 mg of melatonin (△ -2.77, -1.90, and -1.95, respectively). Feelings of Vigor-Activity were also decreased after ingesting 0.3 mg relative to 1.0 mg of melatonin (△ -1.33). POMS responses indicated an increase in self-reported feelings of Fatigue-Inertia, relative to placebo, after ingesting 0.3, 1.0, and 10 mg of melatonin (△ +2.14, +1.56, and +2.28, respectively). Sleep test sleep onset latencies were shorter, relative to placebo, after ingesting 0.1, 0.3, 1.0, and 10 mg of melatonin (△ -7.45, -9.03, -11.02, and -10.32 min, respectively). The duration of sleep (i.e., switch release) experienced during the sleep test was greater, relative to placebo, for the 0.1, 0.3, 1.0, and 10 mg melatonin doses (△ +8.20, +10.04, +12.09, and +10.47 min, respectively). Sleep test self-reported sleep latencies were smaller, relative to placebo, for the 0.1, 0.3, 1.0, and 10 mg melatonin doses (△ -7.60, -10.60, -13.27, and -10.65 min). Subjects also indicated that they slept more quickly after ingesting 1.0 mg, relative to 0.1 mg of melatonin (△ -5.67). Responses to the post-sleep-test SSS indicate that subjects felt sleepier after ingesting 1.0 and 10 mg of melatonin than after ingesting placebo (△ +0.70 and +0.95, respectively). Post-sleep-test SSS responses also indicate that 1.0 mg of melatonin caused greater feelings of sleepiness than 0.1 mg (△ +0.70). Fewer Wilkinson Auditory Vigilance task correct responses were recorded, relative to placebo, after subjects ingested 1.0 and 10 mg of melatonin (△ -4.75 and -5.67, respectively). Correct Four Choice RT response latencies were greater (i.e., longer) after ingesting 10 mg, relative to placebo and 0.1 mg of melatonin (△ +17.21 and +14.46 ms, respectively). All of the pairwise comparison results reported above were significant at the $p<0.05$ level.

Order and Time Effects: Significant order effects were found on the Wilkinson Auditory Vigilance, Simple RT, Four Choice RT, and Symbol Digit Substitution tasks. These results indicate that subjects tended to respond more accurately (Symbol Digit Substitution responses and RT response latencies) with practice and less frequently (Wilkinson Auditory Vigilance) on subsequent test days. These changes are of little interest because: 1) significant treatment-by-order interactions were not found on these measures; 2) the Latin Square is balanced to compensate for order effects; and 3) similar results have been observed previously (14).

There were consistent patterns of variance over time among the mood scale measures. Subjects' SSS [$F(5,75)=17.50$, $p<0.001$] and POMS Fatigue-Inertia [$F(5,75)=8.04$, $p<0.001$] responses indicate that they felt sleepiest and most fatigued, and that they felt the least vigorous (POMS Vigor-Activity scale [$F(5,75)=12.79$, $p<0.001$]) at 1400 h, 2.25 h after melatonin ingestion (Fig. 3). Oral temperatures were consistently low at 1200 and 1400 h (means were 96.85 and 97.09°F, respectively). Mean four choice RT response latencies

were the greatest at 1300 h (i.e., 377.77 ms) and decreased to 360.15 ms at 1700 h. Significant treatment-by-time interactions were found in oral temperature [$F(24,360)=1.60$, $p<0.05$] and number of correct responses on the Four Choice RT task [$F(12,180)=1.92$, $p<0.05$].

DISCUSSION

Ingestion of melatonin (0.1 to 10 mg) at 1145 h resulted in correspondingly increased circulating melatonin levels. Serum melatonin concentrations observed following the 0.1 and 0.3 mg doses were within the normal dynamic range for nocturnal melatonin concentrations (1). Sleep test results indicate that acute administration of melatonin (0.1 - 10 mg p.o.) at midday decreased objective and self-reported sleep onset latencies by an average of 9.46 and 10.53 min, respectively, relative to placebo. Sleep duration was increased by an average of 10.2 min during the 30 min sleep test, relative to placebo, by ingestion of melatonin. Pairwise comparison of SSS scores, following the sleep test, indicate that the 0.3 and 1.0 mg doses of melatonin increased self-reported feelings of sleepiness, relative to placebo, and that feelings of sleepiness following the 1.0 mg dose were greater than those following the 0.1 mg dose. These results indicate that ingestion of an acute dose of melatonin, sufficient to increase circulating melatonin to levels within the normal nocturnal physiologic range, has hypnotic effects. The acute nature of melatonin's hypnotic effect suggests that it may constitute a direct physiologic effect of the hormone independent of its action as a circadian zeitgeber (24-26). This hypothesis is supported by the recent independent observation that melatonin (5 mg p.o.), administered at 1200, 1700, or 1900 h, exerted a direct hypnotic effect, increasing sleep propensity within 90 to 120 minutes of ingestion (27, 28). The phase shifts in circadian rhythms in sleep (27, 28) or endogenous melatonin secretion (29, 30) previously seen after a single acute dose of the hormone, as used here, were of insufficient magnitude to support the hypothesis that the hypnotic effects found in the current study could be caused by melatonin acting as a zeitgeber. Such a hypothesis would suggest that a nine-hour phase-shift in sleep onset can occur following a single acute dose of melatonin at mid-day; this seems highly improbable.

The observed significant decreases in oral temperature following ingestion of 1.0 and 10 mg of melatonin are consistent with previous reports (2,14). Alterations in mood and performance measures following melatonin ingestion, relative to placebo (e.g., increase in feelings of sleepiness and fatigue; increase in Four Choice RT latency; decrease in feelings of vigor, decrease in number of correct responses on the Wilkinson Auditory Vigilance Task), are also consistent in both direction and magnitude of measure with previous reports (13,14). The direction and magnitude of response change, relative to placebo, among the melatonin doses administered were consistent on both the mood and performance measures. It thus seems likely that failure to find significant differences in mood or performance between placebo and the lower melatonin doses (0.1 and 0.3 mg) is due to the limited sensitivity of the measures used, rather than an absence of effect.

The results of this study are consistent with the observations of Vollrath, et al. (6), who report a decrease in daytime latency of sleep onset in subjects given 1.7 mg of melatonin nasally and with those of Lavie and colleagues (27, 28) described above. Nickelsen et al. (31) reported that 50

mg of melatonin, administered p.o. at 0900 or 1900 h caused non-significant decreases in sleep latency, but increased feelings of sleepiness only after the 0900 administration. Others (32,33) reported that evening ingestion of melatonin (1 and 5 mg) did not influence sleep onset or duration, but did cause an increase in REM sleep onset latency. These studies suggest that the magnitude of melatonin's sedative-like effects may be significantly influenced by the time of its administration. Alternatively, the experimental designs used in the negative studies might have precluded observing the hypnotic effect seen here (e.g., by not allowing subjects to modify their sleep times, or by forbidding afternoon napping; 29,30). It should be noted that the pattern of physiologic and performance responses observed here resembles that observed for drugs in the benzodiazepine family (34-36).

In summary, administration of a small melatonin dose (0.1 - 0.3 mg p.o.) during the daytime, which raises serum melatonin concentrations to within the normal nocturnal range, or of slightly higher doses (1.0 - 10 mg p.o.) was shown to cause hypnotic effects, relative to placebo. These effects include a decrease in objective and self-estimated sleep onset latency, an increase in sleep duration, and sleepiness upon waking. Self-reported feelings of sleepiness and fatigue were increased and feelings of vigor diminished. Oral temperature and the number of correct responses on the Wilkinson Auditory Vigilance task were found to decrease significantly following ingestion of 1.0 and 10 mg of melatonin. These results are similar to those reported following ingestion of benzodiazepines and suggest that melatonin may find use as a hypnotic drug. They also suggest that the normal physiologic secretion of melatonin may be an important and direct-acting factor in bringing about sleep onset.

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Figure Legends

Figure 1: Mean (SEM) serum melatonin profiles of 20 subjects sampled at intervals after ingesting 0.1, 0.3, 1.0, and 10 mg of melatonin or placebo at 1145 h.

Figure 2: Mean (SEM) sleep onset latencies, sleep durations, self-reported sleep onset latencies, and post-test SSS responses following ingestion of melatonin or placebo at 1145 h (N=20).

Figure 3: Mean response scores on the Stanford Sleepiness, POMS Vigor-Activity, and POMS Fatigue-Inertia scales throughout testing. Melatonin or placebo was ingested at 1145 h (N=20). Increased feelings of sleepiness, vigor, and fatigue are indicated by higher scores.

Table 1. Mean Differences[+] of Planned Comparisons following Significant Overall F Tests.

	0.0 vs. 0.1, 0.3, 1.0, & 10	0.1 vs. 0.3, 1.0, & 10	0.3 vs. 1.0 & 10	1.0 vs. 10
Oral Temperature	-0.21**	-0.18**	-0.14**	-0.13
Stanford Sleepiness Scale	0.42**	0.25**	-0.05**	-0.01
Profile of Mood States				
Vigor-Activity Scale	-2.02**	-0.77**	0.84**	-0.05
Fatigue-Inertia Scale	1.75**	0.97**	-0.22**	0.72
Sleep Test				
Sleep Onset Latency	-9.46**	-2.67**	-1.64**	0.70
Sleep Duration	10.20**	2.67**	1.24**	-1.62
Self Reported Sleep Latency	-10.53**	-3.91**	-1.36**	2.62
SSS Responses	0.61**	0.48**	0.05**	-0.40*
Wilkinson Auditory Vigilance				
# Correct Responses	-3.72*	-3.03	-2.19*	-0.92*
Four Choice RT				
Correct Response Latency	10.06**	13.25	7.57*	2.62*

[+]Values in table are an average of the subsequent melatonin doses (mg) minus the first dose listed in the heading. All comparisons were F tests calculated with 5 and 15 degrees of freedom, N=20 (see text for details of missing value substitutions), * = $p<0.05$, ** = $p<0.001$.

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Table 2. Mean (SEM) Measured Responses.

	Melatonin Ingested (mg)				
	0.0	0.1	0.3	1.0	10.0
Oral Temperature (°F)	97.55 (0.06)	97.47 (0.06)	97.39 (0.05)	97.31 (0.05)	97.18 (0.05)
Stanford Sleepiness Scale	3.15 (0.10)	3.38 (0.10)	3.66 (0.10)	3.62 (0.12)	3.61 (0.13)
Profile of Mood States					
Vigor-Activity Scale	12.73 (0.59)	11.29 (0.64)	9.96 (0.57)	10.83 (0.65)	10.78 (0.63)
Fatigue-Inertia Scale	3.83 (0.40)	4.85 (0.42)	5.97 (0.44)	5.39 (0.49)	6.11 (0.53)
Sleep Test					
Sleep Onset Latency (min)	17.06 (2.43)	9.61 (1.84)	8.03 (1.60)	6.04 (1.65)	6.74 (1.24)
Sleep Duration (min)	11.36 (2.34)	19.56 (1.79)	21.40 (1.63)	23.45 (1.67)	21.83 (1.41)
Self Reported Sleep Latency (min)	20.55 (2.23)	12.95 (2.07)	9.95 (1.69)	7.28 (1.33)	9.90 (2.07)
SSS Responses	3.80 (0.22)	4.05 (0.26)	4.50 (0.22)	4.75 (0.25)	4.35 (0.26)
Wilkinson Auditory Vigilance					
# Correct Responses	27.30 (1.48)	25.85 (1.32)	24.28 (1.53)	22.55 (1.66)	21.63 (1.56)
Four Choice RT					
Correct Response Latency (ms)	359.41 (8.25)	359.54 (7.36)	367.74 (7.65)	374.00 (9.08)	376.62 (9.86)

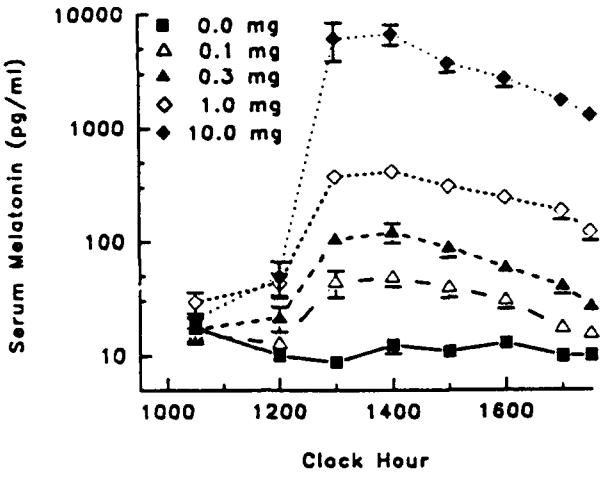

10000
1000
100
10
Serum Melatonin (pg/ml)
0.0 mg
0.1 mg
0.3 mg
1.0 mg
10.0 mg
1000
1200
1400
1600
Clock Hour

Figure 1

Figure 2

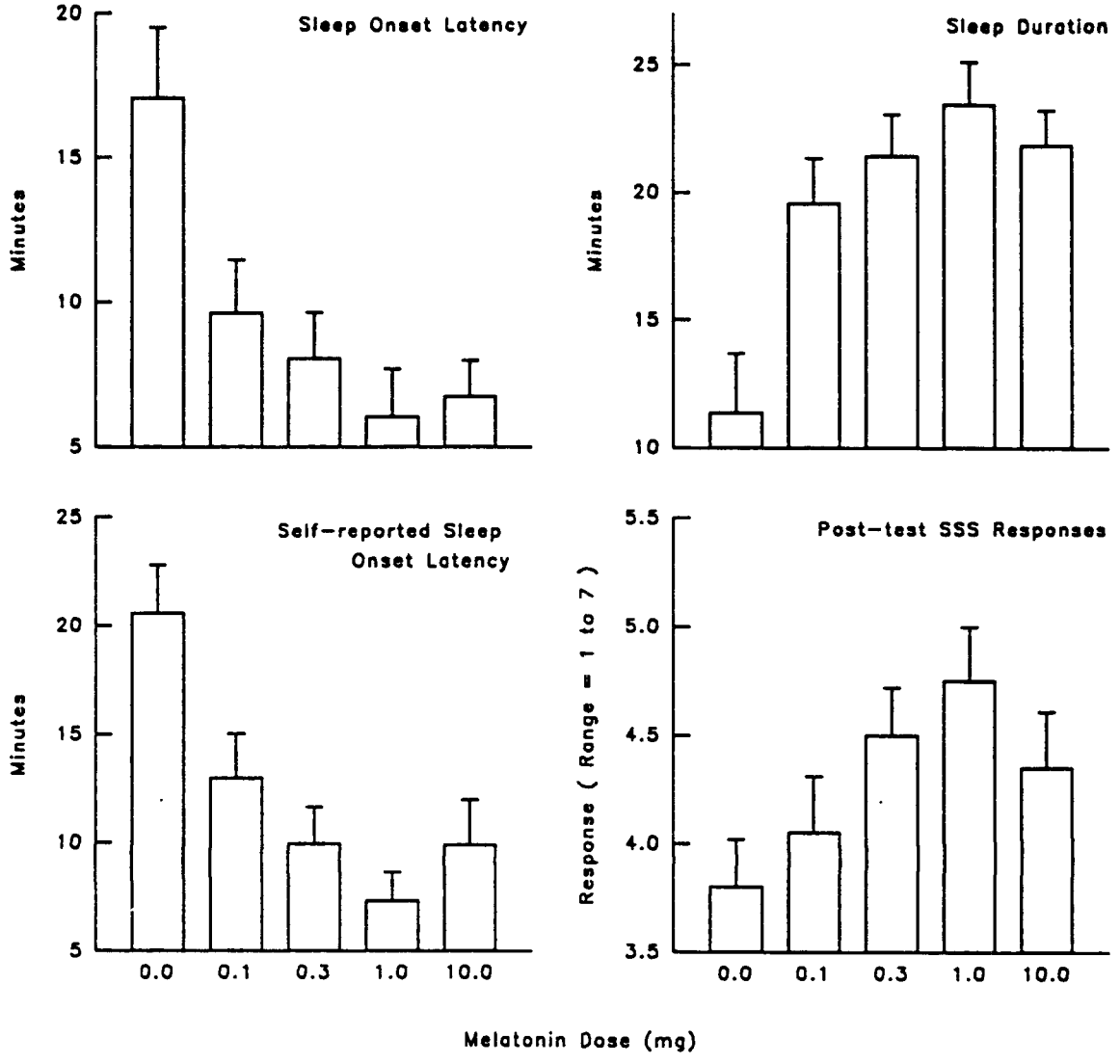
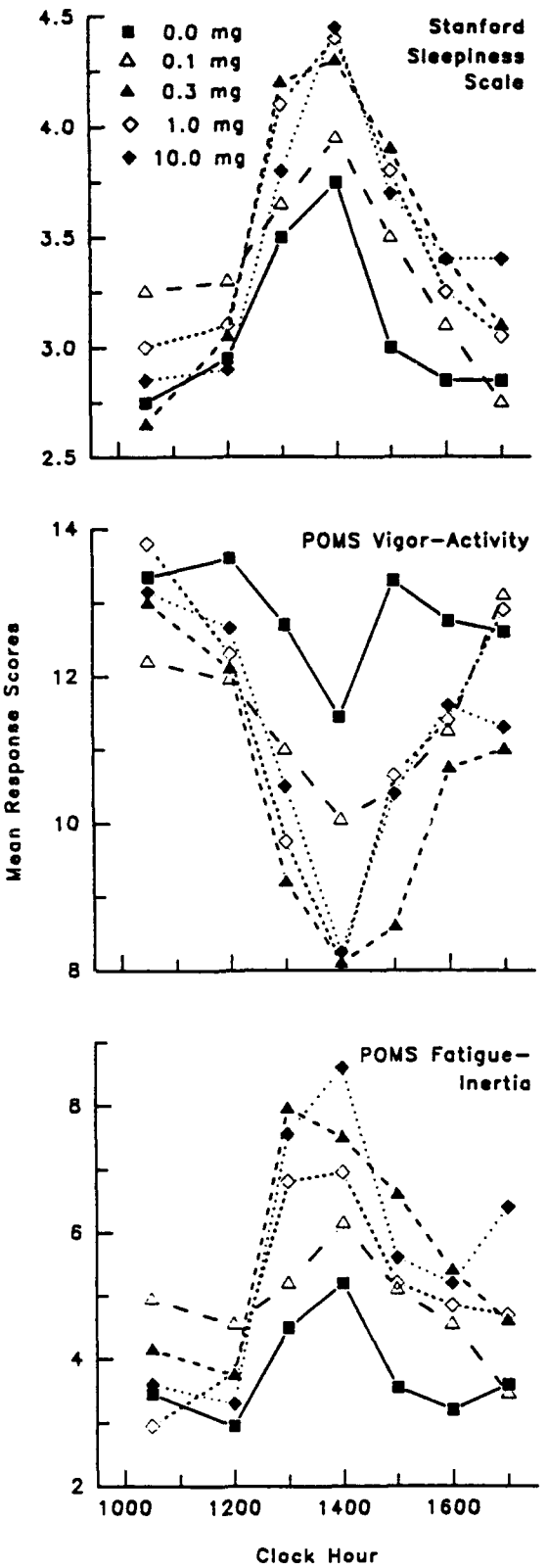

Sleep Onset Latency
Minutes
20
15
10
5
Sleep Duration
Minutes
25
20
15
10
Self-reported Sleep Onset Latency
Minutes
25
20
15
10
5
Post-test SSS Responses
Response (Range = 1 to 7)
5.5
5.0
4.5
4.0
3.5
0.0
0.1
0.3
1.0
10.0
Melatonin Dose (mg)

Figure 3


0.0 mg
0.1 mg
0.3 mg
1.0 mg
10.0 mg
Stanford Sleepiness Scale
4.5
4.0
3.5
3.0
2.5
POMS Vigor–Activity
14
12
10
8
Mean Response Scores
POMS Fatigue–Inertia
8
6
4
2
1000
1200
1400
1600
Clock Hour

Effects of L-Tyrosine Pre-Treatment on Pulse Pressure during Lower Body Negative Pressure Stress

Running Head: L-Tyrosine and LBNP Stress

Andrew B. Dollins**, M.A., Ph.D., Larry P. Krock*, M.A., Ph.D.,

William F. Storm*, M.A., Ph.D., Richard J. Wurtman, M.D.

and Harris R. Lieberman***, M.A., Ph.D.

The Massachusetts Institute of Technology

Department of Brain and Cognitive Sciences

Cambridge, MA

*Armstrong Laboratory

Crew Technology Division

Brooks AFB, TX

Address correspondence to:
Richard J. Wurtman, M.D.
Massachusetts Institute of Technology, Building E25, Room 604
77 Massachusetts Avenue
Cambridge, MA 02139
Tel: (617) 253-6732

** Current address:
DoD Polygraph Institute, Building 3195
Ft. McClellan, AL 36205

*** Current address:
Military Performance and Neuroscience Division
U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760 USA

ABSTRACT

Tyrosine, a large neutral amino acid normally present in protein foods, is the precursor of the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine. Animal studies indicate that systemic administration of tyrosine in pharmacologic quantities can reduce physiological and behavioral decrements induced by highly stressful conditions. This study was designed to test the effects of tyrosine on humans exposed to cardiovascular stress. Twenty participants were exposed to two Lower Body Negative Pressure (LBNP) sessions (-50 mm Hg for a maximum of 30 min) during each of two testing sessions of a repeated measure double-blind placebo-controlled study. The effects of tyrosine detected include: 1) an overall increase in pulse pressure (LBNP typically reduces pulse pressure); 2) an increase in auditory event related potential amplitude (P300-N300), an electrophysiological correlate of attention which may indicate enhanced cognitive activation; and 3) a statistically nonsignificant mean increase of 2.5 min of LBNP tolerance among subjects who were unable to tolerate LBNP for the full 39-min period.

Key Terms:
Electroencephalogram (EEG) Oddball Paradigm
Profile of Mood States Reaction Time

INTRODUCTION

Tyrosine, a large neutral amino acid normally present in protein foods, is the precursor of the catecholamine (CA) neurotransmitters dopamine (DA), norepinephrine (NE), and epinephrine. When systemically administered in pharmacologic quantities, it can, under conditions such as stress, increase brain CA concentration and turnover (9,26,27). Because pure tyrosine is not normally ingested, any dose which significantly increases plasma levels above normal (approximately 70 $\pm$ 3.9 nmols/ml) would be considered a pharmacologic dose. Plasma tyrosine levels peak approximately two hours after oral ingestion of 100 mg of tyrosine and remain elevated for approximately seven hours (10). There are no known adverse effects of tyrosine administration to healthy normal humans. In fact, since it only exerts its effects when a localized deficiency state exists, the effects appear to be system-specific and only present when needed (i.e., when local CA stores are diminished). Therefore, while tyrosine may be more "specific" in its actions than most drugs, tyrosine's effects are likely to be less potent than a drug acting via the same neurotransmitter. Tyrosine availability is rate-limiting for the synthesis of its neurotransmitter products in the brain only when a higher than normal level of transmitter release by catecholaminergic neurons is occurring. When CA neurons are firing frequently and, therefore, releasing more transmitter (DA or NE), they may require more of the precursor - tyrosine, the substrate for transmitter biosynthesis. Frequent neuronal firing enhances the kinetic properties of tyrosine hydroxylase causing this rate-limiting, catecholamine-synthesizing enzyme to be more susceptible to control by this amino acid; it may also deplete the tyrosine pools within nerve terminals (19,25).

Animal studies have demonstrated that tyrosine, given either acutely (in a single dose) or chronically in the diet, reduces adverse physiological and behavioral concomitants of acute stress. For instance, locomotor activity, rearing, and hole-poking behavior were significantly reduced, following 60 min of tail-shock stress in rats pretreated with saline but not with tyrosine (14). Lieberman et. al, briefly review these effects (16).

Studies of tyrosine administration to unstressed humans have demonstrated possible beneficial effects in the treatment of essential hypertension (17) and depression (8). No adverse effects of such treatment were noted (10,15). However, participants in these studies were not subjected to experimental stressors and it is under stressful conditions that tyrosine would be expected to have its positive effects on behavior. We are aware of only one tyrosine study in which human volunteers were subjected to psychologically and physiologically stressful environmental conditions (2). The treatment employed was acute exposure (4 h) to a combination of hypobaric hypoxia (corresponding to 13,800 or 15,500 ft) and cold (60^{0} F). Tyrosine appeared to have robust effects among those individuals who responded most adversely to the stressors on each behavioral task. Many of the decrements in performance, mood, and symptoms induced by these

treatments, including functions believed to be regulated by catecholaminergic neurons, were mitigated by tyrosine treatment. (See Owasoyo, Neri, and Lamberth (20) for a recent review of the effects of tyrosine on stress.)

This study was undertaken to examine the physiological, psychological, and behavioral effects of acute tyrosine administration on normal healthy males exposed to physiological and behavioral stress. Tyrosine administration has consistently produced beneficial effects in animals subjected to various types of cardiovascular (CV) stress (16). It seemed likely that tyrosine would prove effective in reducing the effects of CV stress in humans. Lower Body Negative Pressure (LBNP), was chosen to induce CV stress. LBNP is a technique used to simulate a gravitational load (orthostasis) by exposing the lower body to subatmospheric pressures. This causes blood and interstitial fluids to pool in the lower extremities resulting in decreased venous return and increased sympathetic drive (3). Studies have shown that LBNP induces a variety of hemodynamic changes including increases in heart rate (HR) and narrowing of pulse pressure, as well as decreases in cardiac output, stroke volume, left ventricular ejection time, and venous pressure (11,23). Brief exposures to LBNP (-50 mm Hg) have been shown to increase CA levels (12), suggesting that some of the above CV effects might be attributable to an increase in sympathetic activity. Subjects exposed to LBNP typically respond initially with decreased BP and increased HR. Such changes persist until the CV system is no longer able to maintain homeostatis. At this point, BP and HR rapidly fall and consciousness is lost if exposure to LBNP is not halted.

METHODS

Subjects Twenty-two healthy adult males who had no previous experience with LBNP (mean age = 28.13 $\pm$ 4.71 years) were paid participants in this study. Two of the subjects were unable to tolerate LBNP to -50 mm Hg in at least one of the four LBNP sessions for reasons unrelated to the experimental treatment and were dropped from the analyses. All subjects met the United States Air Force School of Aerospace Medicine (now Armstrong Laboratory) medical requirements for human subjects (as specified by the Advisory Committee on Human Experimentation) and signed an informed consent form before testing (in accordance with AFR 169-3 and the MIT Committee on the Use of Humans as Experimental Subjects).

Procedure A balanced double-blind, placebo-controlled, crossover design was used to control for subject and experimenter bias. The subjects participated in a 1-h training session to familiarize them with the laboratory, procedures, and performance tasks before actual testing. Subjects were then tested, individually, on two occasions separated by at least 7 days. Two 39-min periods of LBNP were included in each 4.5-h test session. Subjects were not tested on Mondays or following holidays to reduce the effects of irregular activity schedules.

Subjects were asked to refrain from alcohol, nicotine, and caffeine consumption for 24 h before testing and to consume only water from 2400 h the night before until their arrival at the laboratory at 0730 h. They were served a breakfast of granola bars and non-caffeinated beverages. Capsules containing tyrosine (50 mg/kg) or placebo were ingested at 0748 and 0900 h. Table 1 provides a schedule of times for events occurring during each test session.

LBNP Subjects were tested while supine, with the lower half of their body in the LBNP chamber. A surgical rubber seal (3-mm thick) was placed around the subject at the level of the illiac crest to ensure air tight integrity. A padded bicycle-like seat was adjusted to prevent the subject from sliding into the chamber when a vacuum was applied. The vacuum source, a Hoover PowerMAX II WET/DRY Vac (Model C2079, Hoover Company, North Canton, OH) was located in a nearby electrically-shielded and sound-attenuated chamber to prevent extraneous noise and electrical signals from interfering with subject testing. Vacuum was applied via a 3.8 cm flexible hose connecting the vacuum source to the LBNP chamber. Pressure was regulated by opening and closing a valve (5.08-cm aperture) mounted on the side of the LBNP chamber. The negative pressure was calibrated with a Hi-Performance Gauge (Model 61A-1D-0800, Wallace & Tiernman, Belleville, NJ) and monitored via a digital display driven by a pressure transducer. As a safety precaution, vacuum pressure was active only while the subject pressed on a positive pressure switch held in his left hand.

During LBNP exposure, pressure was initially reduced to -20 mm Hg for 3 min. Pressure was then reduced an additional 10 mm Hg every 3 min until the internal chamber pressure was -50 mm Hg. Pressure was maintained at -50 mm Hg for the next 30 min or until presyncopal symptoms were observed. The subject's electrocardiogram (ECG) and general condition were monitored continuously throughout each LBNP session. Heart rate and BP (auscultatory cuff) were recorded at least once every 3 min (more frequently if irregular shifts were observed) throughout the LBNP session and until physiological indexes returned to baseline levels. Pulse pressures were calculated by subtracting the diastolic from the systolic BP. Blood samples were drawn: 1) before the onset of LBNP; 2) before LBNP pressure release or as quickly as possible after pressure release; and 3) at the end of each testing session. Behavioral testing continued regardless of when LBNP was returned to the ambient level.

Electrophysiological Measures Heart rate and skeletal muscle activity were monitored throughout the two LBNP periods of each testing session. Data for the initial (baseline through 3 min of LBNP at -50 mm Hg) and final portions of LBNP session 1, all of LBNP session 2, and electroencephalographic auditory event related potential (ERP) sampling were recorded on magnetic tape (Honeywell Model 101, Denver, CO) for off-line analysis. Electrophysiologic signals were amplified using Data Inc. differential amplifiers (Model 2124, Ft. Collins, CO). Electrode impedances were held below 5 K ohms at all sites.

Electrocardiography Silver/silver chloride electrodes (Cleartrace Model 1700-0300, Medtronic Andover Medical, Haverhill, MA) were placed at standard lead II and V configurations throughout testing. Amplifier gains were set to 2 K with high- and low-pass filters set to 0.5 and 50 Hz respectively. Beat-to-beat HR were digitized using a device developed in-house. Average HR were calculated for the baseline before LBNP and every 3 min throughout data acquisition. Eleven data points from strip-chart electrocardiograms were substituted for missing digitized data.

Electromyography Surface electromyographic activity was monitored over the lower left abdomen (rectus abdominus), left thigh (rectus femoris), and right calf (intersection of the gastrocnemius and soleus) to detect tensing of lower muscle groups which could reduce LBNP-induced blood and interstitial fluid pooling. Amplifier gains were set to 10 K and high- and low-pass filters set to 0.1 and 1000 Hz, respectively (60 Hz notch filters active). Cleartrace (Model 1700-0300) electrodes were used. Subjects were asked to relax appropriate muscles when an increase in electromyographic activity was observed (approximately one time per test day).

Event Related Potentials Cortical activity was recorded from 10-20 system Fz and Pz positions referenced to linked earlobes (A1 & A2). The Electro-Cap IX system (Electro-Cap Inc., Dallas, TX) was used to attach electrodes to the scalp. Electrode impedances were checked prior to each recording period. Amplifier gains were set to 10 K and the low- and high-pass filters were set to 50 and 0.5 Hz respectively. The electrooculogram (EOG) was recorded from above and below the right eye using Grass E-5 electrodes (Grass Instruments Inc., Quincy, MA). The electrooculography amplifier high- and low-pass filters were set to 1000 and 0.5 Hz respectively (60 Hz notch filter active) and the gain was set to 10 K.

Auditory ERP activity was recorded using the "oddball" paradigm (24) later off-line analysis. During this paradigm, subjects are exposed to a series of frequent and infrequent stimuli, and are usually asked to count the number of rare stimuli. The P300 wave is a large positive slow wave that occurs approximately 300 msec after the onset of the rare stimuli in the series. The latency and/or amplitude of the P300 wave vary, in a systematic manner, with a wide variety of testing circumstances (6). In this study, subjects were asked to count the number of infrequent tones (1000 Hz) occurring in a series of more frequent tones (2000 Hz) while resting with eyes closed. A total of 330, 50 msec, stimuli were presented at the rate of 1.01 tone/sec. The infrequent tones (20%) were randomly distributed throughout the frequent tones. Subjects were not told the correct number of infrequent tones until all testing was complete. The tones were generated by modifying an Z-200 microcomputer (Zenith Data Systems, St. Joseph, MI) so that the speaker output could be amplified with a SA-150 integrated stereo amplifier (Radio Shack, Ft. Worth, TX). Subjects heard the tones (90 dB SPL) over Sony button earphones which were shielded with sound suppressers to

prevent the impingement of extraneous sounds. A 50 msec stimulus marker was recorded on magnetic tape before the onset of each stimulus, simultaneous with the electroencephalographic and electrooculographic signals. There were two evoked potential sessions during each testing session.

Electrophysiologic data for each trial were sampled at a rate of 512 samples per second for 600 msec. Averaging was time-locked relative to the stimulus marker. Trials with EOG peak-to-peak values greater than 45 uV were omitted from averaging. Many trials contained an artifact corresponding to the QRS complex of the ECG. The data acquisition software was revised to detect the onset of the ECG-QRS complex and to omit averaging of cortical activity for 117 msec following the onset of the QRS complex. The data for subjects with an average of fewer than 30 trials were omitted from further processing. The P100, N100, P200, N200, P300, and N300 peaks and troughs were identified by moving a cursor along each average waveform as it was displayed on a computer monitor. The sequence of average waveforms displayed was randomized over subject and treatment condition throughout this process to prevent experimenter bias during peak and trough identification. Peak minus trough values for the P100-N100, P200-N200, and P300-N300 components of each subject's electroencephalogram (EEG) were calculated for analysis.

Cortisol Levels Blood was drawn from an antecubital vein of the right arm through an indwelling intravenous (IV) catheter (18 g, 3.2 cm, Becton Dickinson & Company, Sandy, UT) capped with a Luer Lock PRN Adapter (Park-Davis & Co, Sandy, UT). Saline (bacteriostatic sodium chloride, USP 0.9%) was injected after each sample was removed to prevent coagulation within the catheter. This technique permitted consistent sampling without the use of a heparin lock. The samples were immediately transferred to chilled centrifuge tubes (containing one ml of ethylenediamine-tetraacetic acid solution) and placed on ice. The plasma and cellular components of the blood were then separated by centrifugation. Two 0.5-ml aliquots of the plasma fraction were stored (-80^{o}C) for cortisol analysis, which was performed using a commercially available radioimmunoassay kit.

Mood Scale The subjects were required to complete three microcomputer based performance tasks and a mood inventory at specific times during each testing session (see Table 1). The software for the tasks was coded in-house and administered using a stock Zenith Z-200 microcomputer equipped with a Gravis Mk VI joystick (Advanced Gravis Computer Technology Ltd., Bellingham, WA) that was connected via a game I/O card (model B107, Magnitronic, Taiwan). The computer display was mounted on a movable stand which was tilted to adjust the display for the subjects' maximum viewing comfort. Subjects responded with their right hand which was positioned along the outside of the LBNP chamber. The performance tests administered were: Dual Task Information Processing, Four-choice Visual Reaction Time, and Simple Reaction Time (16).

A computerized version of the Profile of Mood States (POMS, 18) was administered five times during each testing session. The POMS is a 65 question self-report mood questionnaire that yields six factors when analyzed: Tension/Anxiety, Depression/Dejection, Anger/Hostility, Vigor/Activity, Fatigue/Inertia, and Confusion/Bewilderment. The terms "Flushed", "Light-headed", "Heaviness in legs", and "Sweaty" were added to the usual 65 POMS questions to monitor specific reactions to LBNP induced stress. The additional questions are referred to as the LBNP Stress Factor.

Analyses Except where noted, all analyses of variance were calculated using a between-groups (order), within-subjects (treatment and time), repeated measures factorial design.

RESULTS

Pulse Pressures The mean pulse pressures for which complete data were available (i.e., from resting until six min of LBNP at -50 mm Hg; N = 20) are plotted in Figure 1. Mean pulse pressures (averaged over LBNP sessions 1 and 2) measured during LBNP following tyrosine treatment were significantly greater ($\triangle$ + 3.3 mm Hg) than those following placebo treatment [$F(1,18) = 8.22$, $p < 0.01$]. Mean pulse pressures decreased significantly from 47.8 mm Hg during LBNP session 1 to 42.3 during LBNP session 2 [$F(1,18) = 23.23$,

$p<0.001$]. Pulse pressures (averaged over treatment and LBNP session) also decreased significantly from 52.72 during baseline to 39.76 mm Hg after six min of LBNP at -50 mm Hg [$F(5,90)=28.59$, $p<0.001$]. No significant order (test day) effects or interactions were found. Separate analyses of the two LNBP sessions indicate that the mean pulse pressure was higher when tyrosine was ingested during both LBNP session 1 ($\triangle$ + 3.74, $F(1,18)=5.69$, $p<0.001$) and 2 ($\triangle$ +2.83, $F(1,18)=4.46$, $p<0.05$).

Heart Rate Group mean HR increased significantly from 65.9 beats per min (BPM) during baseline to 80.6 BPM after the first 15 min of LBNP exposure [$F(4,72)=61.30$, $p<0.0001$]. The mean HR during LBNP session 1 was significantly faster [$F(1,18)=26.87$, $p<0.001$] than that during LBNP session 2 ($\triangle$ +4.4 BPM). A significant [$F(1,18)=8.28$, $p<0.01$] treatment by LBNP session interaction was also found, indicating that the decrease in HR between LBNP sessions 1 and 2 was greater when the subjects ingested tyrosine ($\triangle$ -5.68 BPM) than when they ingested a placebo ($\triangle$ -3.06 BPM). No other effects were significant. Separate analyses for the two LBNP sessions indicate HR increased significantly over baseline during both sessions.

Event Related Potentials Sixteen subjects produced artifact-free data for both treatment conditions during ERP session 1 while only eight subjects produced complete data during ERP session 2. The average number of artifact-free trials sampled was 39 per subject (range = 30 to 56). Paired t-tests were used to compare the treatment effects of the P100-N100, P200-N200, and P300-N300 amplitudes for Channels Fz and Pz. A significant [$t(15)=2.13$, $p<0.05$] increase in channel Pz P300-N300 ERP session 1 amplitude of 1.41 uV occurred when subjects ingested tyrosine, as illustrated in Figure 2. A similar though nonsignificant increase of 0.10 uV was observed in Fz P300-N300 amplitude during ERP session 1. No significant treatment differences were found among the P100-N100 or P200-N200 amplitudes of either ERP session or among the P300-N300 amplitudes for ERP session 2.

Cortisol Levels The cortisol levels of ten subjects were analyzed (Figure 3). Five subjects received placebo first and five received tyrosine first. Mean plasma cortisol levels increased significantly from a baseline of 7.11 to 11.29 ug/dl at the end of testing [$F(4,32)=3.48$, $p<0.01$]. There were no significant treatment, order, or interaction effects.

Performance Tasks The number of correctly identified repeated digits on the Dual Task changed significantly over time [$F(2,36)=5.63$, $p<0.007$, $N=20$], but was not affected by the testing order or treatment. Planned contrasts indicate that there was a significant decrease from 15.22 to 13.52 between the first and second test administrations [$F(2,18)=11.67$, $p<0.01$]. No differences were found among subject estimates of the proportion of letters presented or the number of digits erroneously identified as repeated.

Four-choice reaction time (RT) data for two subjects and simple RT data for three subjects were lost through experimenter error, thus only 18 and 17 subjects, respectively, were included in these analyses. A significant increase (+12.2 msec) in Four-choice RT latencies occurred when subjects ingested tyrosine [$F(1,16)=7.81$ $p<0.01$]. The treatment by order interaction was also significant [$F(1,16)=10.69$, $p<0.005$] on this measure. Analysis of the data, divided on the basis of treatment order, indicates that the increase in RT latencies is attributable to the responses of subjects receiving tyrosine followed by placebo [$F(1,7)=28.09$, $p<0.001$] rather than to a treatment effect influencing both groups of subjects. A general performance decrement occurred between the first and last administration of the task as indicated by a significant decrease in the number of correct responses [$F(3,48)=2.805$, $p<0.05$] and an increase in the number of incorrect responses [$F(3,48)=3.55$, $p<0.02$]. No significant changes occurred in the number of premature or time-out errors. Significant treatment by order [$F(1,15)=6.09$, $p<0.02$] and treatment by time [$F(2,30)=4.65$, $p<0.02$] interactions were observed in simple RT latencies. These effects, however, were attributable to a baseline offset which occurred during the 0937 h test. No other differences were observed in simple RT latency or in the number of premature or time-out errors.

Profile of Mood States The subjects' responses on every POMS scale changed significantly as testing progressed. A significant treatment by time interaction was found among the Vigor/Activity scale responses [$F(4,72)=3.11$, $p<0.02$], indicating that subjects ingesting tyrosine felt less "Vigor" during the first half of

a testing session and more "Vigor" during the second half, compared to placebo treatment. The treatment, order, and interaction effects of all other POMS factors were nonsignificant. Response averages indicate that the greatest Tension/Anxiety, Depression/Dejection, Anger/Hostility, Fatigue/Inertia, Confusion/Bewilderment, and LBNP-stress occurred during LBNP session 2, and the least when testing began. Responses also indicate that subjects felt the greatest "Vigor" before LBNP session 1 and the least during LBNP session 2. These differences were significant at the $p < .05$ level, using Duncan's multiple range test.

LBNP Tolerance Of the 20 subjects tested, 9 subjects tolerated LBNP at -50 mm Hg for the full 30 min of testing. The remaining 11 subjects tolerated LBNP an average of 2.5 min longer when taking tyrosine than when taking placebo. Subjects also tolerated an average of 8.76 min more LBNP on the second day of testing than on the first, regardless of treatment. The LBNP tolerance treatment and order effects were nonsignificant, but a significant treatment by order interaction was found [$F(1,9) = 12.91$, $p < 0.005$]. The data were divided on the basis of treatment order and analyzed separately to interpret this significant interaction. Tyrosine treatment significantly increased the LBNP tolerance of subjects receiving placebo followed by tyrosine [$F(1,5) = 6.46$, $p < 0.05$] and decreased the LBNP tolerance of subjects receiving tyrosine followed by placebo [$F(1,4) = 13.50$, $p < 0.02$]. Paired t-tests were used to examine within order treatment effects. Tyrosine treatment increased the average LBNP tolerance of subjects receiving placebo followed by tyrosine from 18.61 to 32.53 min during LBNP session 1 [$t(5) = 4.20$, $p < 0.0085$], but had no effect during LBNP session 2. Tyrosine treatment decreased the LBNP session 2 tolerance of subjects receiving tyrosine on the first day of testing by an average of 9.4 min [$t(4) = 3.77$, $p < 0.02$], but had no effect on LBNP session 1 tolerances for this group.

DISCUSSION

The results of this study indicate that tyrosine treatment reduces physiological decrements attributable to LBNP. Measures which improved with tyrosine ingestion include LBNP tolerance, maintenance of pulse pressure, and channel Pz ERP P300-N300 component amplitudes during the "oddball" task.

Tyrosine treatment caused a significant increase in the duration of LBNP session 1 tolerance of subjects receiving placebo followed by tyrosine, but no difference in the duration of LBNP session 2 tolerance. Subjects receiving tyrosine followed by placebo had lower LBNP session 2 tolerances when ingesting tyrosine but no tolerance differences during LBNP session 1. The average LBNP session 2 tolerances for all subjects differed by 0.47 min ($N = 11$) while the mean LBNP session 1 tolerance was 5.5 min longer when taking tyrosine. As indicated above, all subjects had higher LBNP tolerances on the second day of testing, regardless of treatment. The treatment by order interaction suggests that the subjects experienced more stress on the first day of testing than on the second day.

We interpret these data to indicate that LBNP session 2 was more difficult to tolerate than LBNP session 1 (possibly because the repeated experience of LBNP had a cumulative effect). This is supported by the fact that LBNP session 1 pulse pressures and HR were significantly higher than those of session 2. In addition, self-reported POMS Tension, Depression, Anger, Fatigue, and Confusion, factors were elevated during LBNP session 2. Subjects may have experienced a "first day / novelty" stress which reduced tolerance on the first day of testing. The significant increase in LBNP session 1 tolerance observed in the placebo-tyrosine group may have resulted from tyrosine treatment in the absence of "first day" stress. The nonsignificant increase in LBNP session 2 tolerance was due to the increased difficulty of LBNP session 2. The nonsignificant decrease, with tyrosine treatment, in LBNP session 1 tolerance among the tyrosine-placebo subjects may have occurred because the treatment diminished the "first day" stress. Tyrosine treatment was not, however, sufficient to increase of LBNP session 2 tolerance among these subjects.

The results of this study are consistent with the earlier findings of Conlay et al. (4,5) that tyrosine administration is effective in increasing the BP of hypotensive animals. These studies have shown that tyrosine treatment significantly increases the pulse pressures of subjects, indicating increased cardiac output in response to LBNP. A possible mechanism for this effect is that elevated plasma tyrosine levels lead to an increase in CA, in peripheral sympathetic neurons and adrenomedular cells, and these, in turn, enhanced

cardiac output. These results may also be due to changes in central CA levels. We attribute the significant treatment by time interaction to the same mechanism. While the observed decrease in HR between LBNP sessions 1 and 2 was not expected, it is consistent with our proposal that LBNP session 2 was more difficult than LBNP session 1.

The P300 component of the auditory ERP has been associated with timing or the "intensity" of an information-processing activity (7). In studies where subjects are required to attend to primary and secondary tasks simultaneously, P300 amplitude has been shown to decrease when the "oddball" task is secondary (13). More recent work indicates that P300 amplitude decreases as task difficulty increases (21). Our results of greater P300-N300 amplitude in subjects ingesting tyrosine, relative to placebo, suggest that subjects found the oddball task less difficult when ingesting tyrosine. Arousal and sensory parameters did not change as a function of treatment, as indicated by the lack of significant differences among other peak amplitudes (particularly N100). It is, however, also possible that subjects may have attended and/or responded to more of the infrequently presented stimuli after ingesting tyrosine, causing an increase in average response amplitude that was unrelated to processing ability. While this is, to our knowledge, the first time a direct effect on cognitive activity has been associated with the ingestion of a neurotransmitter precursor, we interpret these results with caution and suggest that further research be conducted in this area.

Data from this human study, which indicate that there were no significant differences in plasma cortisol levels attributable to tyrosine treatment, neither support nor contradict animal research indicating that tyrosine blocks a stress induced rise in plasma corticosterone (22). The current observations are not in agreement with another study (1) which indicated that LBNP has no effect on cortisol levels. This difference in results may be due to the shorter LBNP exposure periods (20 min) of that study.

While responses on the Dual and Four-choice RT tasks, as well as the POMS scales, changed significantly with repeated LBNP exposure and sometimes changed with treatment order, there were no main effects for tyrosine treatment. This result could be attributed to factors other than an insensitivity of these measures to tyrosine administration. The catecholamine depletion experienced during LBNP exposure may not have been severe enough, in either duration or extent, to induce differential responding, attributable to the treatment, on the behavioral tasks used. It is more likely, however, that the activity and distractions which surrounded each subject during testing, and were necessary to ensure his safety, may have obscured any behavioral task differences which might otherwise have been observed.

In summary, the results of this study indicate that tyrosine reduces some physiological decrements caused by LBNP stress. During the first LBNP session, subjects could tolerate longer exposures to this stressor when they received tyrosine prior to exposure. Tyrosine pretreatment also allowed subjects to maintain significantly higher pulse pressures throughout exposure to LBNP, an indication of increased cardiac output. Channel Pz P300-N300 auditory ERP amplitudes were larger in subjects pretreated with tyrosine. We cautiously interpret this as indicating that tyrosine pretreatment enhances central, presumably cortical, information processing and may ameliorate the decrement in cognitive processing ability caused by LBNP. The pulse pressure, HR, behavioral task, and mood surveys indicate that subjects were found LBNP session 2 to be more difficult than session 1. Treatment order effects suggest that subjects reexposed to LBNP a few days after their initial exposure may manifest less psychological "first day / novelty" stress and physiological changes, than during their first exposure.

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Figure Legends:

Figure 1. Average pulse pressures from baseline through 6 min of LBNP at -50 mmHg (vertical bars are SEM, N = 20).

Figure 2. Grand average event related potentials (infrequent trials) for Channel Pz (N = 16).

Figure 3. Mean (SEM) plasma cortisol levels throughout testing (N = 10).

Table 1

Approximate Tyrosine / LBNP Study Time Line *

Start Time	Event	Start Time	Event
0725	Arrive Laboratory	1019	Simple RT 2
0728	Profile of Mood States 1	1025	Profile of Mood States 3
0735	Eat Breakfast	1030	Dual Vigilance Task 2
0748	TREATMENT Dose 1	1042	Blood Draw 3
0820	Enter LBNP Chamber	1045	Begin LBNP Session 2
0822	Insert IV Catheter	1107	Four-choice RT Task 3
0850	Four-choice RT Task 1	1118	Evoked Potential task 2
0857	Blood Draw 1	1124	Profile of Mood States 4
0900	TREATMENT Dose 2	1125	Blood Draw 4
0902	Begin LBNP Session 1	1125	LBNP Off
0907	Dual Vigilance Task 1	1129	Simple RT 3
0937	Simple RT 1	1138	Dual Vigilance Task 3
0940	Profile of Mood States 2	1150	Four-choice RT Task 4
0943	Blood Draw 2	1158	Blood Draw 5
0944	LBNP Off	1158	Profile of Mood States 5
0950	Evoked Potential Task 1	1200	Exit LBNP Chamber
1009	Four-choice RT Task 2	1215	Leave Laboratory

* Task and LBNP start times are actual average times (n=22).

Figure 1

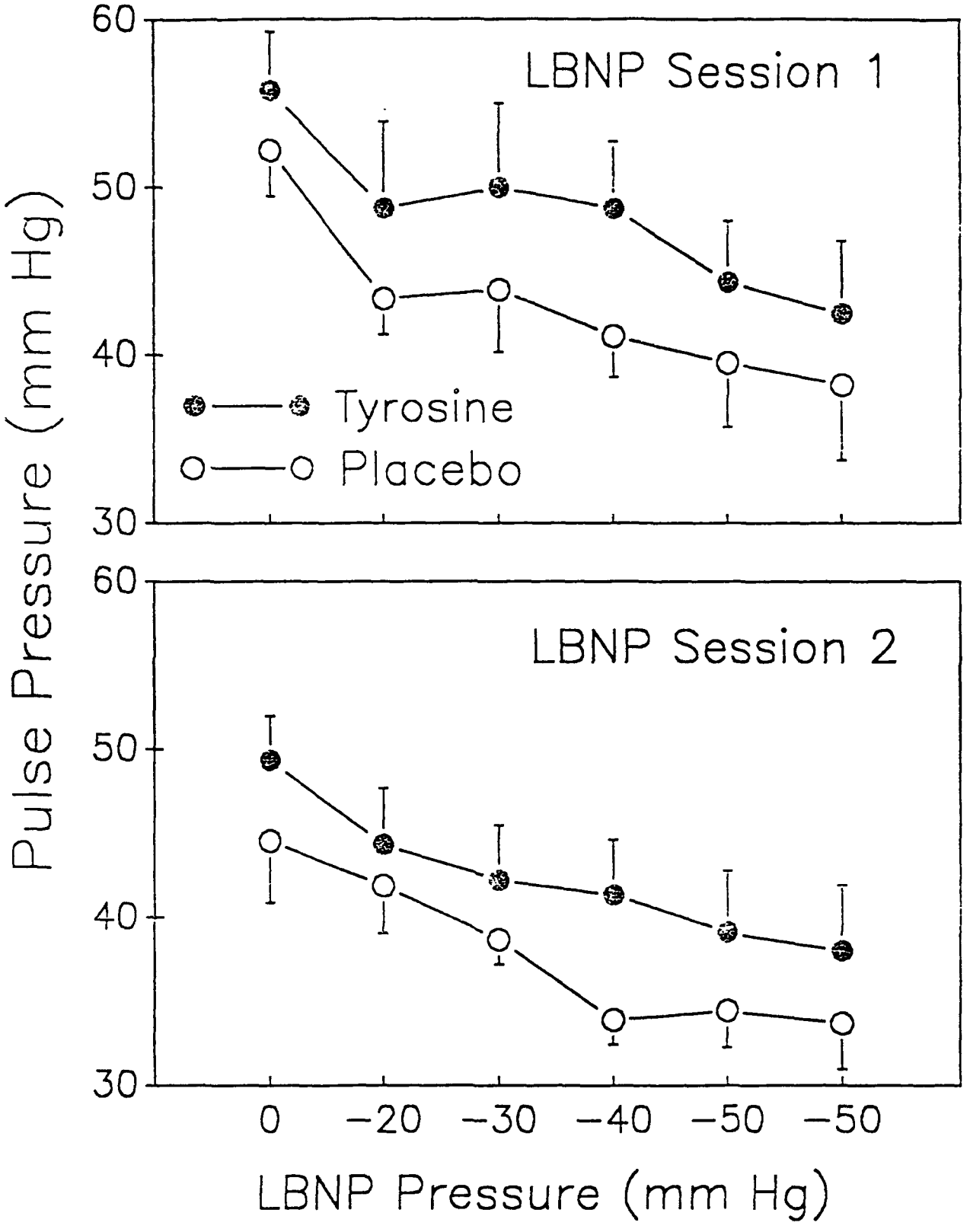

LBNP Session 1
LBNP Session 2
Tyrosine
Placebo
Pulse Pressure (mm Hg)
LBNP Pressure (mm Hg)
60
50
40
30
0
−20
−30
−40
−50
−50

Figure 2

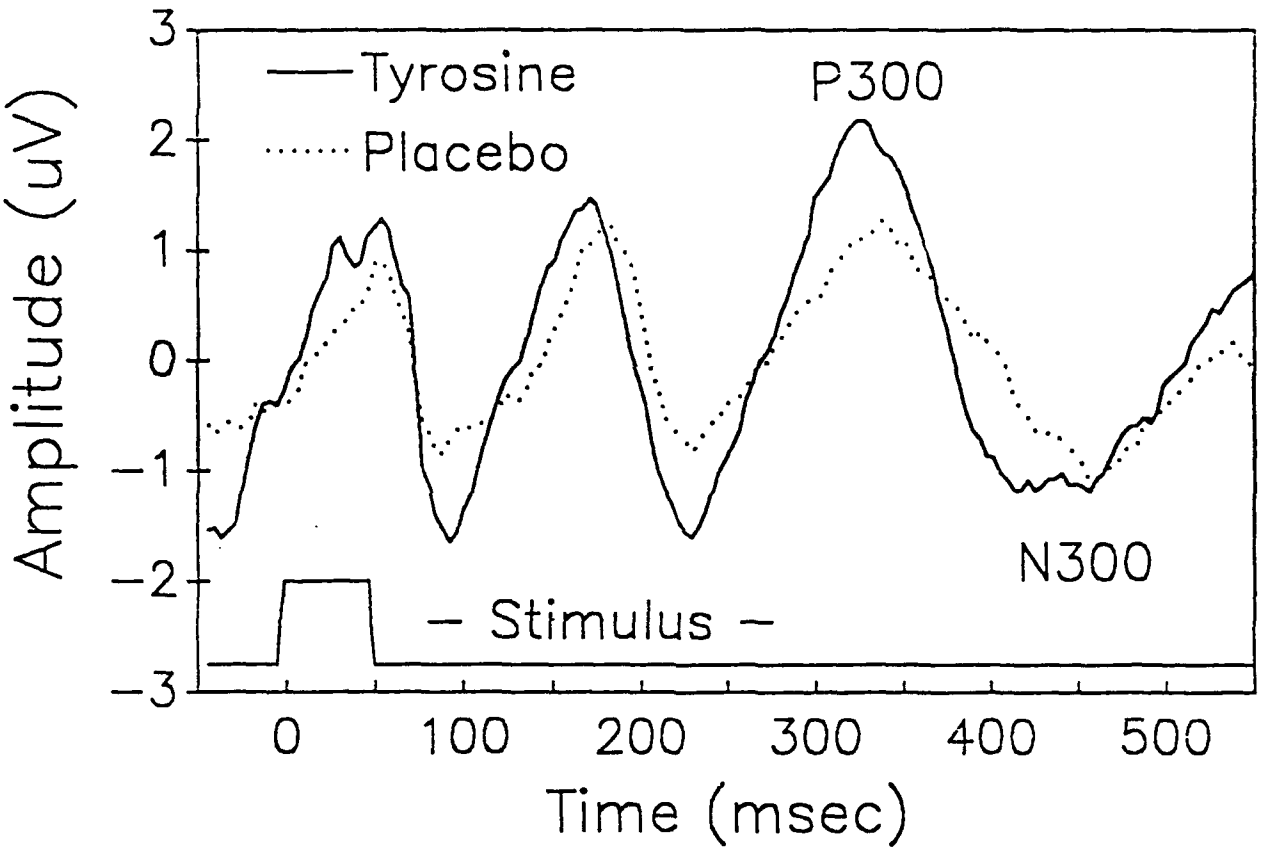
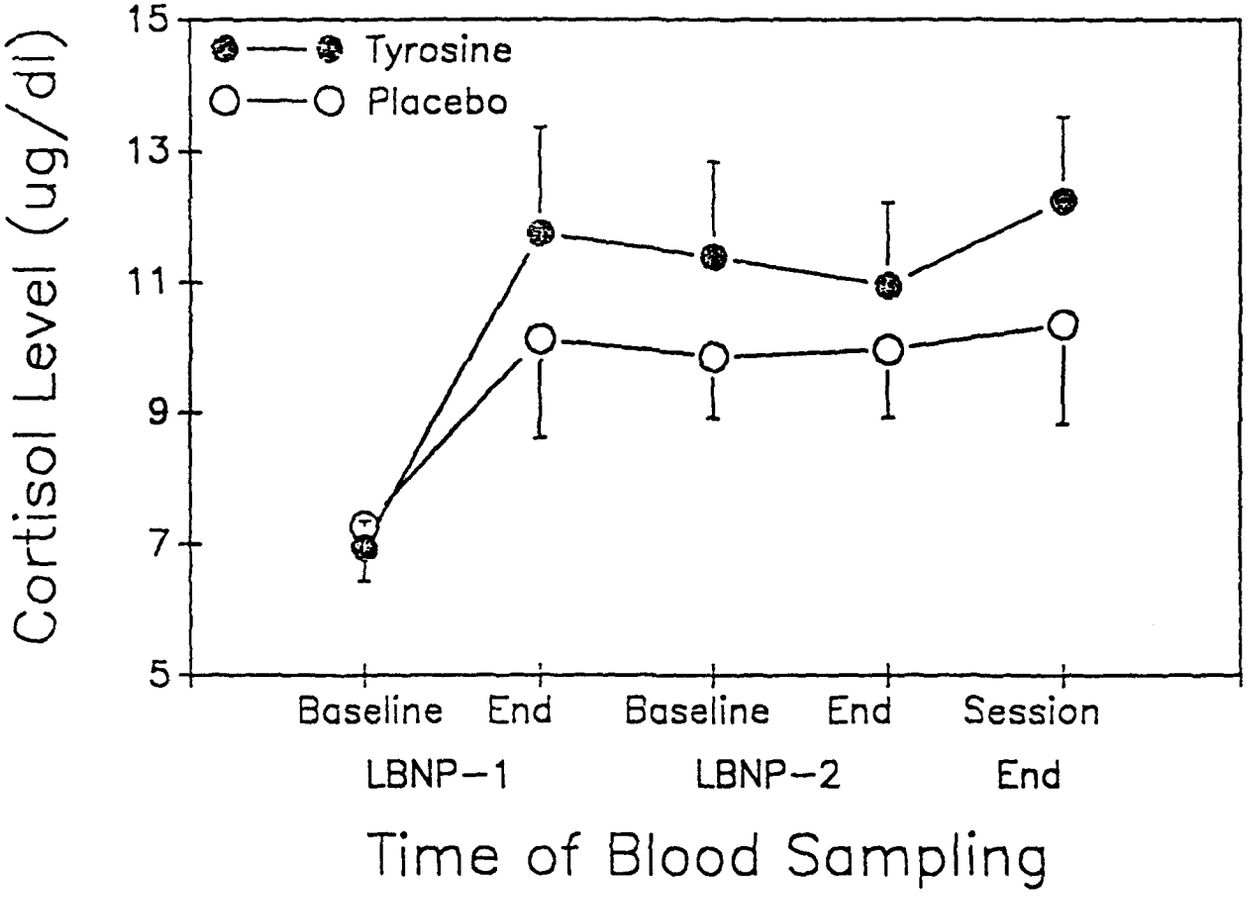

Tyrosine
Placebo
P300
N300
– Stimulus –
Amplitude (uV)
3
2
1
0
−1
−2
−3
0
100
200
300
400
500
Time (msec)

Figure 3


Cortisol Level (ug/dl)
15
13
11
9
7
5
Tyrosine
Placebo
Baseline
End
Baseline
End
Session
LBNP–1
LBNP–2
End
Time of Blood Sampling

Dollins, A.B., Krock, L.P., Storm, W.F., & Lieberman, H.R. (1990). Tyrosine decreases physiological stress caused by lower body negative pressure (LBNP). Aviation, Space and Environmental Medicine, 61(5) (abstract) 491.

TYROSINE DECREASES PHYSIOLOGICAL STRESS CAUSED BY LOWER BODY NEGATIVE PRESSURE (LBNP). A.B. Dollins, L.P. Krock, W.F. Storm, & H.R. Lieberman. USAF-School of Aerospace Medicine, Aero-space Medical Division (AFSC), Brooks Air Force Base, TX 78235 and Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.
INTRODUCTION. Increased plasma levels of the catecholamine neurotransmitter precursor tyrosine reduce the effects of severe stress in animals by enhancing catecholamine synthesis. Similar effects have been observed in humans experiencing mild stress. The current study tested the effect of tyrosine on humans exposed to moderate stress. METHODS. Twenty-one subjects were exposed to two LBNP sessions (-50 mmHg for a maximum of 30 min) during each testing period of a repeated measure double-blind study. Physiological (HR, BP, EEG) and behavioral indices were monitored during testing. RESULTS. Administration of tyrosine (100 mg/kg) was found to: 1) Increase amplitude of the P300 wave - a component of the evoked potential related to information processing; 2) Elevate systolic blood pressure (LBNP reduces systolic BP); 3) Increase LBNP tolerance among subjects who could not withstand LBNP for the full 30 minute period; 4) Decrease depression, tension, and anxiety. CONCLUSIONS. Elevated tyrosine plasma levels reduce physiological and mood decrements caused by LBNP stress.

Dollins, A.B., Lynch, H.J., Deng, M.H., Wurtman, R.J., & Lieberman, H.R. (1991, June 13-14). Effects of ambient illumination on human nocturnal serum melatonin levels and on sustained performance. Paper presented at the annual meeting of the Society for Light Treatment and Biological Rhythms, Toronto, Canada.

EFFECTS OF AMBIENT ILLUMINATION ON HUMAN NOCTURNAL SERUM MELATONIN LEVELS AND ON SUSTAINED PERFORMANCE Andrew B. Dollins, Harry J. Lynch, Mae Hua Deng, Richard J. Wurtman and Harris R. Lieberman Department of Brain and Cognitive Sciences Massachusetts Institute of Technology, 77 Massachusetts Ave. Cambridge, MA 02139

To examine possible relationships between human pineal function and behavior, we monitored, in 23 healthy male subjects, the overnight serum melatonin profile, and performance on a battery of behavioral tasks. Subjects were admitted to the Clinical Research Center in groups of 2 to 4 for a 13.5-hour stay (1630 - 0800 h). On each of three separate occasions, approximately two weeks apart, each subject was assigned to an individually illuminated work station that was maintained throughout the night at approximately 300, 1500, or 3000 lux. (There were two <20 minute snack breaks (2400 and 0400) and hourly toilet breaks; subjects did not encounter light intensities greater than 300 lux during these breaks.)

On admission, a catheter with a heparin lock was established in a forearm vein for withdrawal of blood samples. Throughout the night subjects were required to complete interactive computer tasks designed to measure auditory and visual reaction time and vigilance, and a questionnaire to assess their mood. The task order and times were held constant across test nights. Temperature, blood pressure, heart rate, and sleepiness were assessed hourly. Blood samples were taken from each volunteer at 1900, 2100, 2300 and at hourly intervals thereafter until 0800. Serum was separated by centrifugation and stored frozen until assayed for melatonin concentration by radioimmunoassay.

Repeated measures factorial analyses of serum melatonin levels from 21 of the subjects, assessed by measuring the area under the curve, indicate a significant light treatment effect [$F(2,36)=12.77$, $p<.001$]. Contrasts indicate that the serum melatonin levels under the medium and high intensity lights were significantly lower than under the low intensity ($p<.002$) and that the serum melatonin levels under the high intensity light were significantly lower than those under the medium intensity light ($p<.006$). The light treatment order and light treatment by light intensity effects were not significant. Relationships between serum melatonin levels and the behavioral and physiological measures will be discussed.

Dollins, A.B., Lynch, H.J., Deng, M.H., Wurtman, R.J., & Lieberman, H.R. (1991, November 10-15). Effects of bright light on human nocturnal performance, mood and serum melatonin levels. Paper presented to the Society for Neurosciences, New Orleans, LA.

EFFECTS OF BRIGHT LIGHT ON HUMAN NOCTURNAL PERFORMANCE, MOOD AND SERUM MELATONIN LEVELS A. B. Dollins*, H. J. Lynch*, M. H. Deng*, R.J. Wurtman and H. R. Lieberman Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139. We examined the effects of nocturnal exposure to bright light on human behavior, pineal function, oral temperature and cardiovascular status in 23 healthy male volunteers. On three separate occasions, subjects were admitted to a clinical research center in groups of 2 to 4 for a 13.5-hour session (1630 - 0800 h). Each subject sat at an individual work station that was maintained throughout the night at an illumination of approximately 300, 1500, or 3000 lux. (Three hundred lux is a typical indoor level of illumination). On admission, a catheter with a heparin lock was established in a forearm vein for withdrawal of blood samples. Throughout the night subjects were required to complete interactive computer tasks designed to measure auditory and visual reaction time and vigilance, and a questionnaire to assess mood state. The order and timing of test administration were held constant across test nights. Temperature, blood pressure, heart rate, and sleepiness were assessed every hour. Blood samples were taken at 1900, 2100, 2300 and at hourly intervals thereafter until 0800. Serum was assayed for melatonin concentration by radioimmunoassay. The normal nighttime secretion of melatonin was largely suppressed by the brightest light, partially suppressed by the moderate intensity and appeared to be intact at 300 lux. Mood state was also significantly affected by exposure to bright light. It appeared that bright and moderate light exposure throughout the night increased depression without affecting alertness. However, only subtle variations in performance were noted as a function of light exposure.

Dollins, A.B., Lynch, H.J., Deng, M.H., Kischka, K.U., Gleason, R.E., Lieberman, H.R., & Wurtman, R.J. (1992, October 25-30). Effects of varying doses of exogenous melatonin on human diurnal mood and performance. Paper presented to the Society for Neuroscience, Anaheim, CA.

EFFECTS OF VARYING DOSES OF EXOGENOUS MELATONIN ON HUMAN DIURNAL MOOD AND PERFORMANCE. A.B. Dollins, H.J. Lynch, M.H. Deng, K.U. Kischka, R.E. Gleason, H.R. Lieberman*, and R.J. Wurtman. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139. We examined the effects of 10, 20, 40, and 80 mg of melatonin or placebo, administered at 1145 h, on mood, performance, and oral temperature in 20 healthy male volunteers. Subjects were studied between 0930 and 1700 h on each of five separate occasions. They completed a battery of interactive computer tasks designed to assess performance and mood, the sequence and timing of which were held constant across test days. Temperature was assessed and blood samples were taken at regular intervals. Melatonin concentration was measured by radioimmunoassay. Melatonin levels (area under the time-melatonin concentration curve; AUC) differed significantly in response to the various doses. Mean AUC for the 0, 10, 20, 40, and 80 mg doses were 60, 12228, 27186, 52557, and 106223 pg/ml/7 h, respectively. All melatonin doses, relative to placebo, significantly decreased: oral temperature; number of correct responses on Wilkinson Auditory vigilance; response latency on Four Choice reaction time; and self-reported Vigor (Profile of Mood States [POMS] questionnaire). Melatonin also increased self-reported Fatigue, Confusion, and sleepiness (POMS and Stanford Sleepiness Scale). Differences were not detected among the melatonin doses. Since no temperature, mood, or performance differences resulted from the melatonin doses tested, the dose of oral melatonin necessary to cause significant short term sedative-like effects may be lower than previously believed.

Key Words: Dose-response, Reaction time, Vigilance, Oral temperature

Dollins, A.B., Lynch, H.J., Wurtman, R.J., Deng, M.H., Kischka, K.U., Gleason, R.E., & Lieberman, H.R. (1992, December 9 - 11). Effect of pharmacological daytime doses of melatonin on human mood and performance. Poster presented at the General Clinical Research Center Program Directors Association Annual Meeting, Reston, VA.

EFFECT OF PHARMACOLOGICAL DAYTIME DOSES OF MELATONIN ON HUMAN MOOD AND PERFORMANCE AB Dollins, HJ Lynch, RJ Wurtman, MHDeng, KU Kischka, RE Gleason, & HR Lieberman MIT Clinical Research Center & Dept. Brain Cognitive Science, Cambridge, MA 02139. This study was conducted to investigate the relationship between exogenous melatonin and human behavior. Melatonin (10, 20, 40, or 80 mg, p.o.) or placebo were administered at 1145 h on five separate occasions, to 20 healthy male volunteers and its effects on serum melatonin levels, mood, performance, and oral temperature were monitored. Subjects were studied between 0930 and 1700 h. A battery of interactive computer tasks designed to assess performance and mood was completed, oral temperature was measured, and blood samples were taken for serum melatonin radioimmunoassay. The areas under the time-melatonin concentration curve (AUC) varied significantly in proportion to the various melatonin doses. Compared with placebo treatment, all melatonin doses significantly decreased oral temperature, number of correct responses in auditory vigilance, response latency in reaction time, and self-reported vigor. Melatonin also increased self reported fatigue, confusion, and sleepiness. Since no temperature, mood, or performance differences resulted from the melatonin doses tested, the dose of oral melatonin necessary to cause significant short term sedative-like effects may be lower than previously believed.

Dollins, A.B., Zhdanova, I.V., Deng, M.H., Lynch, H.J., Watkins, C.J., & Wurtman, R.J. (1993, November 7-12). Induced daytime melatonin levels comparable to normal nocturnal levels affect human mood and performance. Paper presented to the Society for Neuroscience, Washington, D.C.

INDUCED DAYTIME MELATONIN LEVELS COMPARABLE TO NORMAL NOCTURNAL LEVELS AFFECT HUMAN MOOD AND PEROFMRNACE.

A.B. Dollins, I.V. Zhdanova, M.H. Deng, H.J. Lynch, C.J. Watkins* & R.J. Wurtman. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

We examined the effects of 0.1, 0.3, 1.0, or 10 mg. of melatonin or placebo, administered at 1145 h, on sleep latency and duration, mood, performance, and oral temperature in 20 healthy male volunteers. Subjects completed a battery of tests designed to assess mood and performance between 0930 and 1700 h.; the sequence and timing of the tests were identical on each day. The sedative-like effects of melatonin were assessed by a simple sleep test: at 1400 hr., subjects were asked to hold a positive pressure switch in each hand and to relax with eyes closed, while relcining in a quiet darkened room. Latency and duration of switch release, an indicator of sleep, were measured. All melatonin doses significantly increased sleep duration, as well as self-reported sleepiness and fatigue, relative to placebo. Sleep onset latency, oral temperature and number of correct responses on the Wilkinson Auditory Vigilance Task were significantly decrased by all melatonin doses. Responses did not differ between the 1 and 10 mg. doses. The 0.1 and 0.3 doses of melatonin produced elevations in circulating melatonin levels comparable in magnitude and time course to normal nocturnal levels. These data suggest that such normal melatonin levels have sleep-inducing properities.

Key Words: Vigilance, Sleep, Oral temperature, Sedative